

# Olfaction in Animal Behaviour and Welfare

Edited by **Birte L. Nielsen**



# 10 The Role of Olfaction in Relation to Stress and Fear

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Like other sensory functions in animals, olfaction contributes to an understanding of the environment; odours include signals that may be considered as threatening and may induce fear and a stress response. These olfactory cues can originate from conspecifics or predators and constitute a wealth of information that is used to promote survival. Chemical signals can be used to assess the presence of unknown conspecifics, possibly competitors for precious resources (food or sexual partners), or predators. Interestingly, olfactory perception and its influence on behaviour are affected by the inner state of the animal via hormones. Here, after defining the stress response and fear, I will review evidence that stressed animals might perceive odours differently. Next, we will see how olfactory signals can generate a stress response and induce fear, and look into experimental support for the claim that odours can be used for stress relief.

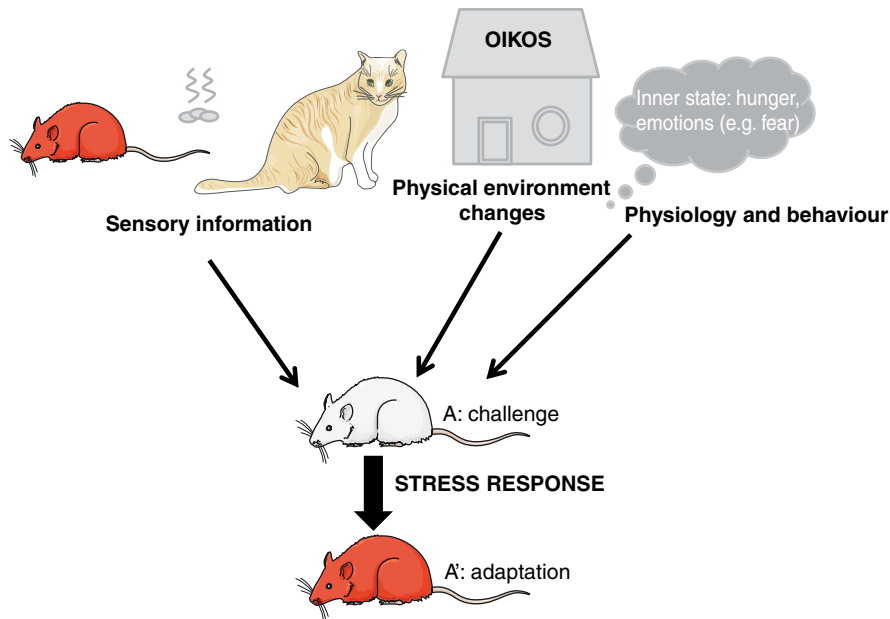
## **Definitions: The Stress Response and Fear**

Bickering about the exact meaning of words is not the exclusive remit of linguists and angry lovers; in order to understand the biology of ‘stress’ and ‘fear’, a few definitions can go a long way. My intention here is to give a brief outline of the stress response in order to understand how it relates to fear and how a stress response can be induced by odours and affect animal behaviour. Research is a dynamic and ongoing process, and I invite readers to refer to more eminent colleagues for good generalist reviews of the literature on stress biology (e.g. Marian Joëls, Stafford Lightman, Bruce McEwen and Robert Sapolsky, to name but a few). It might also be relevant to compare and contrast laboratory findings to those from studies in wild animals; ecological

physiology can enlighten us about the significance of stress (Boonstra, 2013). The word and concept of ‘stress’ is used in material sciences to describe the application of a force onto an object, a strain leading to bending or torsion and eventually breakage. In our everyday life, stress is commonly used to describe the psychological strain resulting from adverse events (Fig. 10.1). Therefore, stress is sometimes also defined as a lack of ‘well-being’ (physical and psychological), but an insight about how this concept was developed will enlighten us further.

### **Hans Selye: the strange case of the clumsy experimentalist**

The application of the word stress to animal physiology is generally (but perhaps wrongly, see Jackson, 2014) attributed to Hans Selye, an Austro-Hungarian scientist working in Montreal around the mid-20th century. At that time in the field of endocrinology (the study of hormones), gland extracts were prepared and injected into laboratory animals in order to observe and understand the effects of hormones. According to the legend, Selye was not very skilled at handling rats and perhaps over the course of the injection treatments, a few were dropped or jumped away from the syringe and had to be chased around the lab. Any proper experiment includes a comparison with a control group in order to allow the identification of the specific treatment effects, in this case rats injected with a neutral substance such as physiological saline. Intriguingly, Selye observed symptoms in both groups: enlarged adrenal glands, smaller immune tissue (e.g. thymus) and the presence of ulcerations in the digestive tract (Selye, 1950). This triad of symptoms turned out to be caused by the chronic experimental disturbance to the rats,



**Fig. 10.1.** If animal A experiences a challenging situation, its stress response allows integrated body and mind adaptation (A). Stressors can be diverse in their nature (e.g. sight, sound and odours) and origin (social signals including competitors, predators), and past experience can influence the animal's reaction.

which seemingly induced an adaptive physiological response, rather than the specific effect of the gland extracts. Selye popularized the notion of stress, following his work on this 'general adaptation syndrome', which is now better known as stress response. Acquisition of scientific knowledge always builds upon previous findings. It is worth mentioning that this model was built on advances from earlier physiologists, such as Claude Bernard who had developed the notion of '*milieu intérieur*' (that the composition of internal fluids are tightly regulated), and Walter Cannon who worked on the regulation of homeostasis through the famous 'fight or flight' response. In experiments that would nowadays be seen as cruel and require much argumentation in terms of research ethics, Selye later showed that noxious physical stimuli (injection of toxins, exposure to cold, haemorrhage, etc.) or stressors that disturb homeostasis induce an adaptive response in animals, and that sustained activation of this initial stress response can lead to pathological states (Selye, 1950). Now we know that stressors also include psychological/emotional insults, such as social isolation, overcrowding, and exposure to dominant individuals in what is known as social defeat paradigms. This is a concrete example of the mind–body relationship,

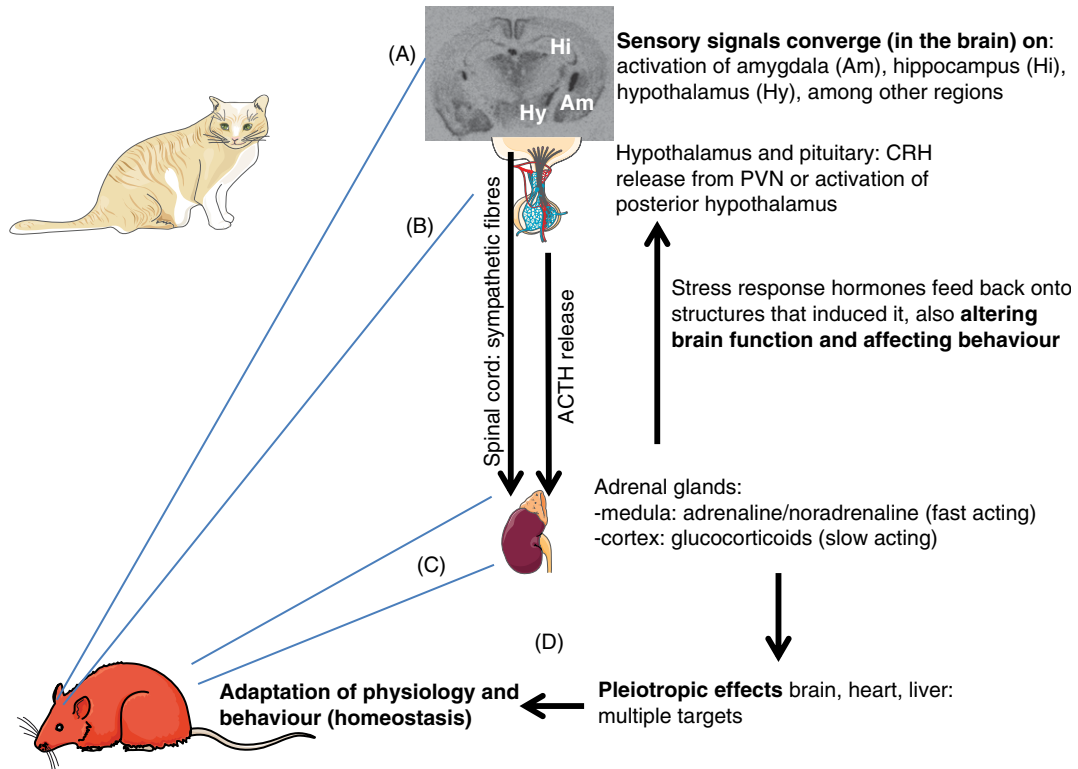
which is easy to manipulate and relatively easy to induce/observe experimentally. Selye's 'general adaptation syndrome' can be divided into three phases: an acute alarm phase involving the sympathetic nervous system (with action of the adrenaline and noradrenaline hormones), a resistance phase where body resources are mobilized to allow adaptation to the stressor (with action of the glucocorticoid hormones) and finally a recovery phase, where resources are replenished. In the case of repeated stimulation or insufficient resources for recovery to occur, an exhaustion phase can follow. This is where the adaptive effects of the *acute stress* (single stimulation) can lead to the pathological effects of *chronic stress* (repeated and inappropriate stimulation of the stress response). In addition to the famous triad of symptoms induced by chronic stress, further consequences have since been reported and will be described below. Those pathological effects of chronic stress often require physiological alterations in order to achieve homeostasis, a process named *allostasis*. Authors therefore refer to the *allostatic load* as the 'wear and tear', the damage caused by chronic stress (McEwen, 2007). In addition to being a scientist, Selye became a prolific popular science writer, spreading his work and his views on stress. It is noteworthy that a

distinction was made between what he named distress (negative stress) and eustress (positive stress), the notion that not all stress is detrimental. Things are not this simple indeed: the arousal caused by confronting a pleasant or positive yet new situation can lead to physiological manifestations consistent with the stress response caused by negative stimuli. Finally, the physiological impact of stress depends on the context in which it is encountered and the state of mind of the individual. When rats are chronically treated with electric shocks, they develop stomach ulcers. By modulating the shock delivery protocol, it is possible to alter stressor predictability or induce helplessness and this affects the development of those ulcers (reviewed in Weiss, 1972). Chronic stressors have a more negative impact if they are unpredictable and if the animal lacks (perceived) control. Now that we appreciate what stress

is, we can ask: what are the manifestations of the stress response and how does it work?

### The stress response: mechanisms

The stress response is a coordinated physiological reaction to threats and environmental change. It is a complex endocrine signalling system. It involves what is known as the hypothalamic–pituitary–adrenal (HPA) axis. In order to help us understand in detail some of the interactions between stress/fear and olfaction, and assess how it can relate to animal behaviour, it is necessary to give a short introduction to the more mechanistic aspects of the stress response. I have tried to keep it as simple as possible; Fig. 10.2 provides brief graphical support for explanations that could otherwise induce fear or stress in you, the reader.



**Fig. 10.2.** Experiencing adversity, a physiological challenge or certain emotions (fear) leads to activation of various brain centres (A), that converge on the hypothalamus (B) whereby signals are sent, in order to activate the adrenal glands (C), to trigger the release of stress hormones. As the hormones act on various targets, multiple body systems are recruited (D) in order for the physiology and behaviour of the organism to be adaptive. Sustained activation of this stress axis can lead to pathologies. ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; PVN, paraventricular nucleus.

When an animal is placed into a stressful situation, a series of events takes place: signals from several brain regions, gathering information about the environment (including the amygdala, see below), converge and act on the hypothalamus. The hypothalamus is a communication centre, a brain region involved in sending and receiving signals between the brain and the rest of the body. Stimulation of the posterior region of the hypothalamus leads to signals activating the adrenal gland medulla via the spinal cord and the splanchnic nerve, causing the rapid release of adrenaline and noradrenaline. The effect of these hormones is called pleiotropic: they act on multiple body systems in order to generate an adaptive response. For instance, the effects of adrenaline on cardiopulmonary function and perspiration are well described (remember this next time you have to speak in front of strangers, with sweaty palms and your heart thumping in your chest). In parallel, the paraventricular nucleus of the hypothalamus (PVN) also receives inputs that lead to secretion of corticotropin-releasing hormone (CRH). This peptide has effects on various brain regions to coordinate the stress response, but mostly signals to the pituitary gland to release adrenocorticotropic hormone (ACTH), which travels through the bloodstream and in turn stimulates the cortex of the adrenal glands to release glucocorticoid hormones (predominantly cortisol in large mammals and fish, and corticosterone in rodents, birds and reptiles). Glucocorticoids also affect the brain to inhibit in turn the signals that lead to their secretion. This negative feedback ensures a tight control over adrenal hormone secretion.

These steroid stress hormones are also pleiotropic and affect virtually all aspects of physiology with a vast range of consequences. From their name (gluco-) we can recognize they will affect the metabolic tissues (liver, fat) to mobilize energy resources (e.g. induce release of glucose for muscles to work), but also the nervous system to provide an adaptive behavioural response. It is the sustained secretion of glucocorticoids that caused the triad of symptoms in Selye's rats and affected both the thymus (lymphatic immune system) and the gastrointestinal tract. Since the nervous system is also a target system affected by this hormonal response, behaviour will be impacted (moods/emotions, decision-making and perhaps – as we will see – perception). Although secreted as part of the same stress response, catecholamine and glucocorticoid hormones have different effects on physiology. If we were to use a running metaphor, the catecholamine response can be compared to what is required to run a 100 m race,

whereas the steroid response is more like what is required to run a marathon. As described above, stressors can be physical factors from the environment (temperature, noise, pollution) or social signals (predators, dominant conspecific). Odours can act as stressors (see below). Captivity can be a stressor for wild and domesticated animals because it reduces their ability to express the full spectrum of naturally occurring behaviours. Certain production practices used on farms can induce stress in the animals concerned, such as tail docking in pigs and beak trimming in poultry, and the effects of these procedures on the animals' stress response are well known (e.g. Prunier *et al.*, 2005; Xie *et al.*, 2013).

### **Who is feeling stressed? How to detect a stress response, and its behavioural consequences**

Even if animals could talk, would they be able to tell us whether they are stressed? Would a human always truly report this? It is sometimes challenging to identify and quantify stress. One difficulty in assessing stress levels is determining and interpreting whether those measures relate to arousal in a positive experience, or a negative stress stimulus that results in less well-being. Physiological markers can be useful but we shall see that behavioural assessments may be more informative as they integrate physiological effects and life experiences.

Most of the aforementioned mediators of the endocrine stress response can be detected and measured in biological fluids. For instance, glucocorticoid hormones (cortisol, corticosterone) are assayed in tissue or body fluids such as blood and saliva. Glucocorticoids are frequently used as stress biomarkers but since they also have circadian and ultradian secretion patterns, they vary during the day in the absence of stressors. Faeces and urine can be alternative sources as they are hormone excretion routes (depending on the species) and integrate secretion over time, smoothing out this variability. Such measures can be used to monitor baseline hormone levels in control animals or investigate longer-term secretion of stress hormones, for instance in animals subjected to chronic stress. Adrenaline and ACTH are less frequently used, as they are quickly secreted and therefore require special sampling techniques. However, physiological manifestations of the early stress response caused by adrenaline (sweating, heart rate or blood pressure, body temperature) can be more non-invasively quantified.

In a world where resources are limited, there is a strong evolutionary pressure to maintain an ability to respond to variations in an ever-changing environment, whether you are a predator (find food) or prey (avoid being someone's dinner). Behavioural effects of stress exposure can be species specific, depending on ecology and evolution, but there are striking similarities across vertebrates. Most importantly there are individual variations, some due to genetics and some due to life experience, which can remain fairly consistent over the animal's life. For instance, several studies report the numerous consequences of early life adversity (social or nutritional stressors, early weaning). Some authors refer to those traits consistent across situations as the personality of an animal. In other words, some animals are more prone than others to fearful reactions (see below) or to the effect of the stress response. A consequence of this variability of stress effects and sensitivity is the difficulty to extrapolate from published data to our own experimental paradigm. The impact of the same stressor can differ enormously, depending on the animals concerned and the environment they live in, and this must be characterized accordingly.

The induction of a stress response will result in various behaviours that can be understood as adaptations to the presence of a real or perceived threat (Greenberg *et al.*, 2002). But as stressor exposure is prolonged, other behaviours – perhaps more pathological – can be expressed. Stress will increase arousal and attention, perhaps leading to changes in stimuli salience. There is a body of evidence suggesting that sensory perception can be modified, e.g. increased for sounds and reduced for pain. If free movement is possible, and depending on whether the location is familiar or not, locomotor and exploratory activities can be induced or repressed. Stress will affect mood, resulting in increased signs of anxiety – these will depend on the ecology and history of the species considered, e.g. in rodents they are manifested by fear of open spaces and freezing (alert immobilization) and more generally by certain types of vocalizations, increased defecation/micturition and fleeing. The intraspecific social relationships (e.g. dominance) might be perceived differently, perhaps causing aggression and fighting between individuals. Besides the many behavioural responses that can be observed following stressor exposure, there are clear effects of stress hormones on memory, including fear-memory, thereby potentially impacting future behavioural

patterns. The consequences for the stress response of altering a set of (stress) hormones will result in alterations in other hormone levels, including reproductive hormones and hormones related to metabolism. In other words, reproductive physiology (fertility, sexual or parenting behaviour) and metabolic physiology (metabolism, appetite and ingestive behaviour) can also be impacted by the induction of the stress response (for an in-depth review, see Greenberg *et al.*, 2002). The behavioural effects of the acute stress response can thus be extensive.

The behavioural effects of chronic stress can appear from as early as 2 weeks of exposure in animal models, depending on the stressors applied. Chronic stress can cause alterations in behaviours, sometimes similar to acute stress as discussed above, and the repeated exposure to stress hormones affects numerous brain systems. This will impact motivation, affective behaviour and mood. The appearance of depressive-like symptoms and altered mood-related behaviours (anxiety disorders) are among the more recognized consequences of chronic stress (McEwen, 2007). In humans, depression is a persistent feeling of sadness and loss of interest, an ill-defined nebula of symptoms, diagnosed via the reported persistence of some of those symptoms for a certain time. In the most severe cases of major depressive disorders (MDDs), symptoms can sometimes be alleviated with a number of drugs. However, MDDs are only one extreme of the full range of mood disorders and these are possibly harder to cure than to define and identify, but advances have been made using animal models. In laboratory animal models of depression, two behavioural traits, corresponding to the human depressive symptoms, are usually studied: despair and anhedonia. Despair, or learned helplessness, is a behaviour pattern that develops in animals that have been exposed to aversive stimuli or noxious situations they could not escape or overcome, and therefore were unable to express the stress response's fight or flight. This is often assessed in laboratory rodents using the tail suspension test (Steru *et al.*, 1985) or the forced swim test (Porsolt *et al.*, 1977), whereby immobility (hanging still or floating, respectively) is considered a sign of resignation to adversity (behavioural despair). Anhedonia, loss of pleasure seeking behaviour, can be studied in tests where a choice is given between a pleasurable stimulus and a more neutral one, e.g. water or sweetened water, where loss of preference for the sweet water is considered to be anhedonia-like (Katz, 1982). More recent and more refined

paradigms involve testing choices in the animal, with a view to assess cognitive bias along the lines of the famous image: is this glass half-empty or half-full (Harding *et al.*, 2004; Brydges *et al.*, 2012)? Depressive-like and anxious disorders are often associated with other behavioural changes such as inhibited social behaviour (Beery and Kaufer, 2015), alterations in self grooming (Kalueff *et al.*, 2016) and increased irritability or aggressiveness (Neumann *et al.*, 2010). Finally, at least in humans, chronic stress leading to allostasis is not solely a risk factor for the development of affective disorders, it also acts as a trigger that promotes the development of other psychiatric conditions such as bipolar disorders or addiction (Pettorruso *et al.*, 2014). Clearly there is progress to be made on the understanding and assessment of the pathological effects of long-term stress on animals, but interesting patterns are emerging.

### Fear

Emotions are cognitive and physiological alterations in an individual that result from exposure to certain environmental or social signals. Fear is an emotion that is generally induced by detection or even suspicion of a danger in the environment. Fear behaviour will manifest differently according to the ecology of the species concerned. For rodents it is the smell of predators and being in open spaces where they might feel vulnerable, whereas equids might seek open spaces where they are more likely to see predators. Fear responses include behaviours that promote danger avoidance (e.g. increased vigilance or anxiety-related behaviours) and physiological readiness for the 'fight or flight' response (Nettle and Bateson, 2012). Fear is transient by nature, often associated with activation of the amygdala region of the brain, which will result in stress responses (see above); sustained exposure to stimuli that induce fear can sometimes affect physiology and behaviour in ways consistent with chronic stress exposure.

Odours are powerful sensory cues when it comes to fear: they can generate fear reactions in an acquired/learned and innate fashion. In a classical (Pavlovian) negative conditioning paradigm, odours can be used as the conditioned stimuli: a neutral olfactory cue is administered with a noxious stimulus. Following several repetitions, learning occurs and a pairing is made between the cue and the noxious stimulus. Finally, the neutral olfactory cue

alone can elicit a fear/stress reaction (expectation of noxious stimulus). Interestingly, fear learning generally occurs with high glucocorticoid levels (which are known to affect memory), indicating an interesting area of interaction between those phenomena.

Certain odours can create an innate fear reaction: without prior exposure to the olfactory stimulus, spontaneous fear and stress reactions can be induced, such as seen in some prey species exposed to predator odours (see below). This trait would be highly favoured through the course of evolution, and avoidance of potential danger could be seen as homologous to the ancestral fear some of us have of the sight of snakes or spiders (though it is difficult to unravel the innate from the cultural in humans). It should be noted that numerous odorant compounds become aversive at high concentration and can induce avoidance behaviours due to their unpleasantness.

### Olfaction is Affected by Stress

Our perception is affected by our internal state. I despise the bakery shop next to the train station, fanning out the tempting smell of fresh croissants every morning, because it is a daily reminder that I cannot eat *ad libitum*. Hunger or satiety make us react differently to an appetizing food smell, since feeding-related hormones (e.g. insulin) affect olfactory function (see Chapter 6). The same goes for the effects of our stress hormones (catecholamines, CRH, ACTH, glucocorticoids such as cortisol or corticosterone) whose secretion has profound impacts on physiology and behaviour. Hormones act on receptors, with the presence of receptors in a cell type or a tissue reflecting the potential for responsiveness to a given hormone. Several brain regions associated with olfactory responses (e.g. the olfactory epithelium and bulb) express such receptors for adrenaline, CRH or glucocorticoids, suggesting olfaction can be impacted by exposure to stressors and that altered olfaction could indeed be part of the coordinated body response. Given that sensory inputs can be affected by the inner state of the animal, one can hypothesize this might have evolved in order to optimize adequate perception, maximize decision-making and enhance survival. There are conflictual early reports in rodent models, where animals were adrenalectomized and olfactory performance was either reduced (Sakellaris, 1972) or unaltered (Doty *et al.*, 1991). More recent

research brings some elements of response to the question of how stress might impact olfaction.

Acute stress has been reported to impact aspects of olfactory perception. It has been shown that acute injection of a synthetic glucocorticoid (dexamethasone) acts on the olfactory mucosa, where the first step of olfactory detection takes place (Raynaud *et al.*, 2015b). This pharmacological treatment mimics the glucocorticoid stress response and appears to induce a more acute sense of smell in a rat model. In another study, noradrenaline released during a mild stressor exposure has been shown to act on the olfactory bulb to repress the formation of olfactory memories in an odour recognition task (Manella *et al.*, 2013). This suggests that a mildly stressed animal may not remember odours so well, because stress hormones could directly affect the olfactory bulb. Other studies show quite the opposite for strong stressors and trauma-associated odours in mice (Jones *et al.*, 2008). When an electric foot shock was associated with an odourant molecule (acetophenone) for which the olfactory receptor is known (M71), this led to increased freezing in the presence of acetophenone alone after 3 weeks of training (ten pairings per week), which is characteristic of Pavlovian conditioning. Intriguingly, this was accompanied by an increased number of M71 neurons expressing the cognate receptor to this odourant, suggesting increased sensitivity to acetophenone. This morphological change is an example of experience-dependent plasticity in the olfactory system, induced by associative learning (McGann, 2015). It seems that acute stressors or aversive learning can impact olfactory function in animals, sometimes rapidly but also more slowly (tissue reorganization); those findings require further validation in other models in order to be generalized to species other than rodents.

Repeated exposure to stressful situations has been shown to affect olfactory perception. Chronic stress has profound effects on metabolism or the immune system, which can shape and affect the substratum for animal behaviour (the brain) and any epithelia of the body (including the olfactory epithelium). Classically, stress is known to affect brain structures involved in emotion processing, memory formation and fear: the prefrontal cortex, the hippocampus and amygdala (fear-associated region). But new work shows olfactory regions can be affected. We recently demonstrated that the first step of olfactory detection was reduced in the olfactory epithelium following

a regimen of chronic variable stress (unpredictable stressors), where rats developed depressive-like symptoms (Raynaud *et al.*, 2015a). These findings were later extended to the olfactory bulb, where mice chronically treated with corticosterone in their drinking water developed anxious and depressive-like disorders (Siopi *et al.*, 2016). This was accompanied by changes in cell survival in the olfactory bulb and a loss of olfactory discrimination, acuity and memory at the behavioural level. Those two studies on the negative impact of chronic stress on the olfactory system in rodent models mirror a wealth of data from human patient studies. A range of effects have been reported in depressive patients, from loss of olfactory sensitivity (Buron and Bulbena, 2013) to alterations in the pleasant sensation brought by odours (olfactory anhedonia; Naudin *et al.*, 2012). Qualitative and quantitative effects on olfactory perception are interesting to study in humans, because we can accurately describe how we feel and smell, something that we can only infer from animal studies. In another patient population (people suffering from panic disorders; Wintermann *et al.*, 2013), brain imaging studies showed that brain regions were differently activated in response to odours, suggesting changes in the way the information is processed. Interestingly, Krusemark *et al.* (2013) showed this also to be the case in healthy subjects, in which a transient anxious state is induced: different brain regions were activated and the interactions between the regions were also different. Those last studies confirm that although odour perception was not found to be affected, the brain processes in response to odour presentation differed. This supports the idea that emotional and behavioural responses to odours can be affected by stress. Finally, it is worth mentioning that in animal models where olfactory sensitivity is reduced through genetic modification of the olfactory detection machinery, anxiety and depressive-like effects can be detected in rodents (Glinka *et al.*, 2012; Chen *et al.*, 2014) and fish (Abreu *et al.*, 2016).

From a hormonal point of view, it is not just the stress hormones described above that are released in response to stress exposure, a number of other hormonal systems can be stimulated or repressed. Chronic stress can affect the reproductive axis, for instance by decreasing testosterone production in males, disrupting the ovulation/oestrous cycle in females and even altering sex-specific behaviour in many species (Toufexis *et al.*, 2014). These are



examples of some of the indirect effects of the pervasive stress response. Olfactory loss in animals appears to be comorbid with symptoms of chronic stress; this may manifest in reduced feeding, social abilities or reproductive function. Animal management practices should take into account these effects, in species and situations where odours represent important sensory information.

In summary, olfaction is affected by hormones of the stress response. Acute stress, both through general arousal and specific olfactory mechanisms, modifies the salience of olfactory stimuli and alters their perception and memorability. Chronic stress, often in association with depressive-like symptoms, is associated with losses in olfactory perception.

### Odours Can Induce a Stress Reaction

Stressed animals might perceive odours differently, and odours themselves can induce a stress response. Although olfactory cues are potent conditioned stimuli in associative learning, I will only focus here on how fear and stress reactions might be induced in an innate fashion.

#### Fear from predator odours

Strong unpleasant smells can assault our senses and make us feel uncomfortable. Butyric acid is the product of fatty acid degradation by bacteria and is well known for smelling like rancid butter or sweaty armpits. Whether you or your neighbours on the train suffer from a lack of fridge or deodorant, you might avert your nostrils from the source or even breathe through your mouth (and risk retinal olfaction). When rats are allowed to explore a test arena containing an odorant source in a corner, they appear to avoid spending time near butyric acid and also avoid 2,4,5-trimethyl-3-thiazoline (TMT), a component of fox faeces odour (Endres and Fendt, 2009). TMT has an unpleasant smell, but unlike butyric acid it also causes an increase in the amount of time the rats spend freezing. Freezing is a transient immobilization whilst the rat appears alert, thought to help reduce harm in threatening situations and classically recognized as a fear-related behaviour. This shows that it is not only unpleasantness that drives seemingly innate fear behaviours, but that certain compounds specifically induce a fear reaction (see Chapter 2).

Historically, laboratory scientists have induced fear in rodents using a variety of odours sourced

from diverse predator species (cat, puma, wolf, fox): cloth rubbed on fur, used bedding, anal gland secretions, faeces and urine (Apfelbach *et al.*, 2005). Further field studies of prey reaction to scary predator odours include a large bestiary of farm and wild animals from all over the world (Apfelbach *et al.*, 2005). Generally, these innate fear reactions can also be induced by purified compounds identified in secretions from predators; for instance, TMT (as above) and various pyridine analogues (Brechtbuhl *et al.*, 2015) including 2-phenylethylamine (PEA; Ferrero *et al.*, 2011). There is evidence that the fear-inducing properties of predator smell are dependent on their meat-based diet (Berton *et al.*, 1998; Osada *et al.*, 2015). Cats are carnivorous and yet some French scientists still managed to feed one a vegetarian diet. The same animal was subsequently fed freshly killed mice and in both cases the faeces were collected (Berton *et al.*, 1998). Next, mice were tested in an exploratory task, and the meat faeces caused a reduction in exploration and induced more anxious-like behaviours. Smelling a digested fellow mouse in faeces form might be scary. Likewise urine from wolves fed melon did not cause the same avoidance reaction as urine from predators fed meat in four prey species: the mountain beaver (*Aplodontia rufa*), the house mouse (*Mus musculus*), the deer mouse (*Peromyscus maniculatus*) and the guinea pig (*Cavia porcellus*). Is it because feeding melons to meat-eating predators must be somewhat disturbing for them? Or most likely, because the odorant compounds are meat-derived metabolites? Urine odours are a complex mixture of signals indeed. Nevertheless, the captive vegan wolf is not so mighty in the eye of the rodent prey.

Behavioural responses of rats to TMT (Endres *et al.*, 2005) and other predator odours (Apfelbach *et al.*, 2005) have been extensively reviewed elsewhere; these responses include freezing, avoidance behaviour, defensive burying, reduced eating and changes in the acoustic startle response (reaction to a sudden loud noise). TMT odour exposure also leads to a stress response, as evidenced by increases in blood pressure and plasmatic corticosterone levels. This stress response will in turn affect various biological functions and behaviours. The neural pathway of TMT-induced fear and stress responses has been recently described in detail (Kondoh *et al.*, 2016). Upon odour detection, the olfactory bulb is directly connected to a brain nucleus related to the amygdala, the amygdalo-piriform transition area,

which in turn activates the PVN of the hypothalamus and induces the endocrine stress response (corticotesterone production). Several structures dedicated to odour recognition are involved in fear-related molecule detection (Takahashi, 2014): the main olfactory epithelium, the vomeronasal organ or the Grueneberg ganglia (Brechbuhl *et al.*, 2013, 2015). Different brain regions will also be activated by various fear-inducing odorants and this may translate to slightly different physiological and behavioural responses (Takahashi, 2014).

Responses to frightening predator odours are modulated in health and disease. Several studies point to the role of past experience. For instance in cows, the response to wolf urine odour (together with auditory stimulation of recorded wolf howling) was increased in animals bred in areas inhabited by wolves, in comparison to naïve animals that had not experienced wolves (Cooke *et al.*, 2013). In other ungulates, the heart rate of elk (*Cervus elaphus canadensis*) exposed to various predator odours was highly variable between individuals (Chabot *et al.*, 1996). One could hypothesize that these variations are the product of genetic variability (e.g. in emotional or endocrine responsiveness) and life experience (e.g. previous stress exposure). The effect of life experience also applies to attempts to repel rodents using odour sources: they can become less effective, as there is evidence of habituation following repeated exposure to commercial prey repellent (Apfelbach *et al.*, 2005). As seen in Chapter 2, it has been reported that infection with the parasite *Toxoplasma gondii* can lead to alterations in olfactory perception of fear-related odours. Infected rats might become less fearful of cat odours (Vyas *et al.*, 2007), thereby leading to higher chances of an unfortunate final meeting with the predator and increased transmission of the parasite. Science is exciting because some results may force the reassessment of what we think we know: the whole *Toxoplasma* mind-control story might not stand up to scrutiny according to some authors (Worth *et al.*, 2013) and might be debatable.

Interestingly (and perhaps frustratingly for those concerned), there is evidence that TMT or cat urine does not always induce freezing or avoidance in laboratory and field conditions (Apfelbach *et al.*, 2005). Failure to observe the expected result can sometimes be explained. There are species differences in fear responses to TMT, for instance between Sprague-Dawley, Long-Evans and Wistar rats (all laboratory rat strains), the latter being less responsive in terms

of freezing (Rosen *et al.*, 2015). Could it be that, after generations of captive breeding in small plastic boxes, no selective pressure for predator avoidance has led to a reduction in the innate fear trait? Odours are also notoriously difficult to experiment with, as delivery is difficult to assess (has the animal already smelled the test odour?), the odorant concentrations are difficult to control (depending on room temperature or air movements), and the experimental paradigm used will strongly affect the results (size of the experimental arena in which odours are presented, distance to the odour source, illumination); thus, experimentation on fear-related odours can be a scarily complex affair for scientists.

### **Stress communicated from conspecifics**

Empathy can be seen as a complex process only encountered in humans (possibly not even all of us), but it can be homologous to the emotional contagion observed in many animal species. Farmed pigs have been reported to show signs of emotional contagion, both for positive and negative emotions (Reimert *et al.*, 2013). Several laboratories have reported that a stress response can be induced by exposure to odours from stressed conspecifics. This olfactory based ‘empathy’, a form of chemical communication, can be seen as advantageous, in order to alert individuals to possible threats experienced by others.

In an experiment with pigs, when a food dispenser was sprayed with urine from a restrained animal, the latency to feed from it was longer than when sprayed with control urine (Vieuille-Thomas and Signoret, 1992). This suggests pig urine might contain an alarm pheromone, transmitting negative experiences to naïve sows. Studies in cows show they also appear to detect stress in a conspecific. Using feeding suppression as a measure of stress, naïve individuals exhibited a longer latency to feed in the presence of urine from a heifer subjected to electric shocks (Boissy *et al.*, 1998). The smell of stressed heifer urine also induced a rise in cortisol levels in the exposed animals. Faeces may also contain such stress signals. It has been shown that rats can distinguish between faeces from stressed and non-stressed conspecifics (Valenta and Rigby, 1968; Mackay-Sim and Laing, 1980). However, chemical signals are not all contained in urine and faeces. Exposure to various stressors has been shown to cause the production of an airborne signal inducing

a number of physiological and behavioural stress responses in naïve rats (Kiyokawa *et al.*, 2013). Even in anaesthetized rats, electric stimulation of the perianal gland led to the production of this alarm pheromone (Kiyokawa *et al.*, 2013; Inagaki *et al.*, 2014). This social signalling of arousal in animal groups is perhaps an evolutionary strategy in order to rapidly propagate information that can be crucial to survival and those odorant molecules are the volatile homologues of sonic alarm calls.

What is the nature of such odorant alarm molecules? Sometimes they can be compounds with pheromone properties (Chapter 3). In the case of the rat alarm pheromone, a mixture of two compounds (4-methylpentanal and hexanal) is reported to be responsible for the information transfer (Inagaki *et al.*, 2014). In other cases those compounds can be evolutionarily ‘borrowed’ from other species. For instance, stressed mice secrete a sulfur-containing volatile compound that shares chemical features with compounds from meat-eating predator urine (2-*sec*-butyl-4,5-dihydrothiazoles). Mice use it to signal non-specific dangers to others, as this alarm signal is detected by the Grueneberg ganglia (Brecht *et al.*, 2013).

Where do these chemical signals come from? As seen above they can be produced by various glands. Additionally, adrenaline might induce sweating, causing body odour (skin/hair bacteria), thereby leaving chemical cues about our past (and perhaps present) emotional state. Faeces and urine are frequently sources of such odorants, which might be the reason why increased defecation/micturition are observed in cases of stress, perhaps under the influence of stress mediators (and perhaps the reason for the expression encountered in several languages that links defecation and fear). Finally, chronic stress alters immunity at the level of epithelial tissue (e.g. digestive tract or skin), thereby altering the qualitative and quantitative properties of the microbial communities living there and therefore the odorant metabolites they release. It is conceivable that chronic stress might be signalled or detected through olfactory signals.

Stress odours may have other uses than communicating distress to conspecifics. In an extensive survey of frog secretions, green tree frogs (*Litoria caerulea*) are reported to emit a nutty odour from their parotid gland when stressed (Smith *et al.*, 2004). It is not known whether conspecifics are impacted; it is thought to serve as an aposematic deterrent, a strong signal to deter and scare predators away.

One can wonder whether this odour causes stress in their predators. Finally, I mentioned earlier the notion that not all stress is detrimental, and a rise in cortisol levels may simply reflect arousal caused by a novel situation. Studies of African wild dogs (*Lycaon pictus*) in a zoo show that an endocrine stress response can be induced by exposure to prey odour such as gazelle and that this could be used for enrichment purposes (Rafacz and Santymire, 2014), although see Chapter 13 for potential pitfalls in the use of odours in zoos. From a practical perspective, this knowledge should be incorporated into animal husbandry and care practices. Perhaps potential stressful signals should be washed away, in order to avoid communication of distress states that would generate a stress response (e.g. see Chapter 14 on the effects of odour removal on the behaviour of laboratory rodents). There might be a huge animal welfare potential to harvest from an improved knowledge of the odorant signal semantics, which could potentially allow us to show more empathy to animals in our care.

### **Odours that Alleviate the Effects of the Stress Response?**

If odours can induce a stress response, could there be odours that reduce it? An open mind allows you to embrace ideas you’d never considered before, but – to paraphrase the comedian Tim Minchin – never be so open-minded that your brain falls out. Nowadays, we believe we can treat the pathological manifestations and consequences of inappropriate or exacerbated stress responses. Several pharmacological agents can act with an alleged high specificity on targets in the nervous system, affecting adrenalin effects or serotonin levels, for instance, to reduce anxiety or depression. Beta blockers are known to reduce the effects of adrenal catecholamines on physiological and psychological responses to acute stress. Although they are not always effective at the human population level, selective serotonin reuptake inhibitors (SSRI, aka Prozac and other trademarks) are reported to alleviate in some cases the depressive symptoms often precipitated by chronic stress. In the past and present, other cultures with differing views of the medical process have used inhalation aromatherapy for diverse ailments. Several scientific studies point towards a role for odours in the amelioration of stress in various experimental models. Many plant-derived products have remarkable pharmacological

effects when used topically or internally. Here, I will solely discuss inhalation of odorant compounds. Is there a way to manipulate the stress response and its effects using odours?

Oils extracted from citrus fruits have been a popular source of odorants in stress-relief research. Essential oils from bergamot, *Citrus bergamia*, have positive effects on anxiety and depression symptoms (Navarra *et al.*, 2015). Patients waiting for their dentist appointment are an interesting population in which to study the potential beneficial effects of odours (Lehrner *et al.*, 2000). Men and women were asked to fill out questionnaires about their inner state and emotions while sitting in the dentist waiting room, in the unannounced presence or absence of orange oil (*Citrus sinensis*). Men were not affected by the presence of odours, but female patients reported less anxiety, a more positive mood and a higher level of calmness. Such effects of odours in humans could be attributed to the aesthetic element of odour exposure, perhaps helping humans dealing with adversity and awaited loss of control of sitting in a dentist chair. There is anecdotal evidence that odour exposure during therapy sessions can help patients suffering from post-traumatic stress disorder (PTSD; Daniels and Vermetten, 2016). This might have grounding and calming effects, a distraction from the negative experience of reliving a traumatic experience. Animals cannot fill out questionnaires about their feelings, but can be tested in the lab with anxiety and depression tests in response to odorant exposure. Rats were subjected to tests (Porsolt *et al.*, 1977) in which they were forced to swim in a beaker of water for 15 min on day 1, then re-immersed in a beaker of water on day 2 and their swimming activity quantified. Duration of immobility, i.e. the time spent floating by the animal, was measured during the day 2 swim episode. This reflects learned helplessness, as the floating is assumed to indicate the rat has given up attempting to escape. Learned helplessness is a symptom of depressive disorders, and it is widely used to detect anti-depressant activity of pharmaceuticals. Rats that were exposed to lemon oil vapours between days 1 and 2 showed reduced immobility time, a marker of anti-depressant activity (Komori *et al.*, 1995). This reduction in immobility time was confirmed independently in mice (Komiya *et al.*, 2006). Lemon oil also had anxiolytic effects in an elevated plus maze task (Komiya *et al.*, 2006). In this latter study, lavender and rose oils did not produce any

anti-depressant or anxiolytic effects. However, rose oil had anxiolytic effects in rats in another report (de Almeida *et al.*, 2004) and was also shown to reduce the endocrine corticosterone induction response (Fukada *et al.*, 2012).

While their colleagues were busy testing essential oils from all sorts of fruits, others worked on the effect of another odour source: a mixture of *trans*-2-hexenal and *cis*-3-hexenol called green odour (GO), which can evoke in humans the smell of fresh cut grass. GO has been reported to have anti-stress properties in laboratory rodents for a wide range of experimental paradigms. In models of prenatal stress, application of stressors to gravid dams sometimes leads to depressive-like behaviours in offspring (Fujita *et al.*, 2010). Following 10 consecutive days of forced immobilization for an hour in a restraint tube during the second half of gestation, dams gave birth to offspring that developed abnormal stress responses (as measured by plasma markers) and depressive-like symptoms. Offspring from a parallel group of stressed females treated with GO during the stress exposure regimen did not develop such stress-related traits (Fujita *et al.*, 2010), suggesting GO countered the effect of restraint stress. In an experiment where rats were subjected to a daily 3-min forced swim test for 10 days, leading to increased immobility times, GO exposure re-established locomotor behaviour, suggesting a loss in learned helplessness (Watanabe *et al.*, 2011).

As seen above, exposure to the component of fox odour TMT or an electric foot shock can induce an endocrine stress response and increased anxiety in behavioural tests. Rats pre-exposed to GO before TMT or electric foot shock treatment showed a lower stress response than non-GO-exposed conspecifics (Nikaido *et al.*, 2011). Finally, to resonate with the human example mentioned earlier, rats subjected to a fear conditioning paradigm aimed at modelling PTSD showed ameliorated symptoms following GO exposure (Nikaido *et al.*, 2016).

It seems surprising that the claim of an odorant-based therapeutic strategy for acute and chronic stress relief has not been taken up by a larger population of the scientific and medical community. Most of the scientific work published on this topic in the reputable peer-reviewed scientific literature is suitable by scientific standards. The devil is in the detail. There are inconsistencies in terms of protocols used: diversity of control odours, e.g. triethyl citrate (Fujita *et al.*, 2010) and butyric acid (Watanabe *et al.*, 2011) or distilled water (Nikaido

*et al.*, 2011), diversity of behavioural effects and endocrine endpoints. It is conceivable that in certain situations, the presence of an odour could act as an environmental enrichment during a stressful situation, in an otherwise relatively barren experimental cage. There are inconsistencies as to what odours have what effect (e.g. rose oil has anxiolytic effects in some reports but not others). The Cochrane collaboration concentrates on reviewing medical data in order to critically assess the efficacy of treatments and promote evidence-based medical care. A quick search through the Cochrane reviews (comprehensive large-scale meta-analysis studies) indicate the evidence for any effect of inhalation aromatherapy is at best 'equivocal' (if not ineffective) for alleviating suffering in dementia and in labour pain management (Smith *et al.*, 2011; Forrester *et al.*, 2014). Absence of proof is not proof of absence, but these analyses are not supporting measurable effects of inhalation aromatherapy. Overall, in naïve laboratory animals that have never experienced a peaceful walk in the fresh-cut grass, some odors are reported to have appeasing properties. The lack of solid reproducible evidence warrants caution in generalizing this effect.

There are other possible avenues to explore. If animals can learn a negative association with an aversive stimulus (e.g. foot shock and odour) that induces a stress response, it is also possible to learn a positive association with a pleasant stimulus. Would a manifestation of this phenomenon be that animals that can be trained with this positive conditioning would exhibit an attenuated stress response to mild stressors? This could be used for instance to improve welfare of captive animals undergoing veterinary procedures, limiting their stress response. This positive conditioning has been shown in certain cases for pigs (Oostindjer *et al.*, 2011). Young animals whose mothers had been fed flavoured food during late gestation and lactation showed lower behavioural and hormonal signs of stress response upon weaning when the same odour was presented (see Fig. 6.4). It would be interesting to study whether other challenging situations can be made less stressful through this seemingly comforting role of odour application.

In line with what proponents of alternative medicines (such as inhalation aromatherapy) support, there might be a case for the use of odours in management of adverse and pathological effects of the stress response. A challenge in contemporary science is about overcoming positive publication bias

whereby negative data cannot be easily published. More research is needed into what could be a fascinating therapeutic avenue. If real, this potential should be studied, understood and harvested, and if fictitious, the myth should be debunked. Open your mind, but be sure to apply your critical scientific thinking.

## Conclusion

A spontaneous adaptive response takes place in animals subjected to changes in their environment, whether a perceived or a real threat. This stress response can affect olfactory perception and sometimes leads to the generation of odorant signals via bodily secretion, a form of chemical communication. Olfactory cues from other species can elicit a fear or stress response in a naïve animal. Perhaps as a species that does not often use olfaction as our primary sensory modality, we humans might lack some empathy towards animals that do. In order to improve the welfare of captive animals, we should take these aspects of sensory perception into account.

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CABI TOURISM TEXTS

2nd Edition

# Food and Wine Tourism

ERICA CROCE AND GIOVANNI PERRI



## chapter 6



# Food and Wine Tourism Best Practice: Case Studies from Around the World

### LEARNING OBJECTIVE

- To ‘travel’ experiencing some of the best practices from around the world: wineries, dairies, breweries, olive mills, distilleries and others.

Studying the theory behind what constitutes good business practice is fine, as long as its application brings concrete and effective results. Therefore, this chapter is entirely dedicated to looking at a selection of food and wine enterprises that have successfully incorporated tourism hospitality into their product. The case studies set out below range from large international brands to small producers and have been selected by the authors based on their own personal experience of the producers’ professional approach and efficient organization. Within the following pages, food and wine producers in particular (but also students of gastronomic tourism and other operators in

the sector) will find many new ideas to help them to plan, develop and organize tours to welcome visitors to their wineries, farms and other food or wine enterprises. For those who already organize and manage gastronomic tours, it is hoped that the cases below will serve as a valuable benchmark.

In the analyses, each production sector is represented by at least one case study with an explanation of how each different phase of the tour is organized. In some examples, alternative ideas for tours have been added to increase tourism potential and to give a more complete idea of possible visitor management and organization.

### AT THE WINERY

Californian wineries have always been the leading edge as far as tourism hospitality is concerned.

As way back as the beginning of the 1990s, in a country considered by Italians as being totally lacking in gastronomic culture, American winemakers were already demonstrating their great skills in welcoming visitors to their cellars and estates. Because they found themselves having to popularize a wine culture in a country that knew very little about the product (unlike France or Italy where wine has always been part of everyday life), they had little option but to open their doors to visitors in order to make their product known. Not only did they fling their cellar doors open, they already had restaurants on their premises, gadgets in their gift shops and all kinds of events going on.

(Petrini and Padovani, 2005)

## The visit

One winery in particular stands out for its commitment to reaching out to the general public: the Robert Mondavi Winery in Oakville, Napa Valley, California. They have been welcoming visitors almost since the first day they opened in 1966 and over the years they have developed a rich and lively education programme. Their approach is clearly stated on their website:

Educating the American public about wine, food and the arts has always been part of the philosophy behind Robert Mondavi Winery. The winery was one of the first to present educational tours and programs, culinary events, concerts and art exhibits and its visitor programs have become world-renowned.

([www.robertmondaviwinery.com](http://www.robertmondaviwinery.com))

## Organization

The Mondavi winery can be visited and experienced in a number of different ways. Visitors can choose to go on a straightforward tour of the wine cellars followed by a tasting session, or those with more knowledge of wine can opt to do a technical tasting course. They can explore the nuances of food and

wine matching over lunch or enjoy a sumptuous evening meal and vertical tastings of the winery's most prestigious wines in an exclusive and suggestive setting. The choice is extensive.

- Winery tours and tastings: 'Signature tour and tasting' (maximum 15 persons, pre-booking essential); 'Discovery tour' (max. 20 persons, children welcome); 'Wine tasting basics' (max. 15 persons); 'Exclusive cellar tasting' (maximum 10 persons); 'Twilight tour' (max. 15 persons).
- Wine and food experiences: 'The Harvest of Joy tour and lunch' (max. 10 persons); 'Garden to table' (max. 10 persons); 'Exclusive cellar' (max. 10 persons); 'Wine and chocolate' (max. 10 persons); 'Pairing with cheese' (max. 10 persons); 'Four Decades' (6–10 persons); 'To Kalon Room tastings'.

The most complete tour of the winery, which covers every aspect of wine production, is extremely popular and pre-booking is highly recommended. An entry fee is charged and tours take place at fixed times.

- The tour follows the grape's journey from vineyard to bottle:

this in-depth winery tour follows the path of the grape from the vineyard to the cellar to the finished wine. Guests walk into the To Kalon Vineyard, visit the winemaking cellars (including fermentation and barrel aging) and receive a seated educational tasting of three wines with one of our wine education specialists.

([www.robertmondaviwinery.com](http://www.robertmondaviwinery.com))

- Maximum number of participants: 15.
- Children under the age of 13 are not accepted on this particular tour, but other child-friendly tours have been devised to keep younger visitors happy.
- Tour length: about 90 minutes.
- Groups are also welcomed during the grape harvest.
- Information about the winery (original founders, company history, distribution

and markets, etc.) is given during the tour but not in any particular order.

- The wine educator's approach is friendly and informal.

### Arrival and welcome

- The winery is immediately recognizable from a distance as visitors will already have visited the winery's website to book the tour.
- The names of the tour participants are registered at the Visitor Welcome Center.
- Great attention has been given to the visitor areas: elegant and stylish interiors in keeping with the setting and atmosphere of a leading Californian wine estate; beautifully maintained lawns and vineyards, contemporary art pieces, fountains and shady trellises on the outside.
- As individual visitors arrive before the appointed time of the tour, the tour guide (in casual clothes) greets them and introduces himself and gradually gathers everybody together into a group.
- Once everyone is present, the group is formally welcomed in a small room furnished with a few chairs set in a semi-circle and maps on the walls, clearly visible to all. The maps serve as a vital visual support to the opening explanations about the Californian winegrowing areas, focusing on the Mondavi estate. The geographical and climatic features that make it such an ideal territory for wine-growing are described, before going on to explain the concept of *terroir*. The type of language used is simple and to the point, and only goes into more technical explanations once it becomes clear from tour members' questions that they would like to have more detailed or specific information.
- Winemaking is described as an 'outdoor sport' and wine is likened to a newborn baby that needs to be cared for, step by step, as it grows and matures.

### Further ideas

- Different meeting points around a wine estate can be set up for tour groups, particularly if they have booked in advance. One winery that was visited gathers people together under a centuries-old oak tree just by the winery entrance.
- Individual visitors might like to have the opportunity to visit a winery without a guide; this also frees staff from being explicitly engaged to accompany groups. Armed with an audio-guide, information leaflets and interpretive panels to guide them around the estate, tourists can enjoy an independent visit before joining a tasting session led by an expert from the winery. Individual visits such as these represent a great saving on human resources, but possible problems can arise when people have a lack of respect for their surroundings. Careless visitors have been known to damage buildings or equipment and some have even been known to steal things; in fact one of the tasks of a winery guide is to keep a discreet eye on visitor behaviour.
- Individual visitors are well catered for (audio-guides, information leaflets, etc.) at the Château de Meursault (Burgundy, France). In Calistoga, California, the Clos Pegase Winery offers visitors a self-guided art tour. The winery complex was designed by renowned architect Michael Graves in 1987, after winning an architects' competition sponsored by the winery's owners with the San Francisco Museum of Modern Art. The visitor, guided by a leaflet similar to a museum guide, can wander in the garden and admire the proprietor's personal art collection. The idea of building a 'temple to wine and art' was first conceived back in the 1980s. The garden, indeed the whole complex, including the fermentation area and wine tasting rooms, was created as a space for design and leisure: there are over 1000 pieces of original artwork,

with sculptures, paintings and antiques, all celebrating the affinity between art and wine. There is also a Resident Artist programme giving artists the chance to stay on the premises and create. The architectural project:

... responds in grand but playful splendour to the need to be showy and ostentatious. Even the name of the winery, the origin of which comes from a work in the collection representing the myth of Pegasus, was a symbolic choice on the part of the proprietors who wanted to create a connection between their wines and the Greek myths. In doing so, they have laid claim to a nobler and more ancient tradition than their main competitors in the market: French wines.

(Chiorino, 2007)

## Outside

- After the initial welcome and explanation, the group goes outside into a small, protected area facing rows of vines. Visitors are invited to sit on the benches provided for the second part of the talk.
  - The vines in this area have been planted for educational purposes only and each row represents a different species of vine, all of which are grown on the estate. The guide picks a small bunch of grapes from the first row, hands it to one of the visitors and invites them to take a grape and pass it on so the others can do the same. This gives everyone in the group the chance to discover what that particular type of grape tastes like before being made into wine. In the meantime, the guide outlines some of the pedoclimatic features of the *terroir* where the grape is grown, before going on to describe the grape's characteristics, how that particular variety is used and the wines that are made from it. The same is repeated for each of the vines, so that visitors can sample the grapes at different stages of maturation and get an idea of the taste of each variety.
- The atmosphere is relaxed; people are obviously very involved, they ask questions spontaneously and begin to exchange comments among themselves. It is a moment of great interaction. This particular visit takes place around the time of the grape harvest, which is probably the most interesting season for wine tourists; however, it is still possible to give visitors a direct experience throughout the year. Tour guides can, for example, point out the phase of berry growth at that particular time, explain pruning methods and so on.
- Before the group enters the wine cellars they are invited to try a variety of grape that was brought to California by the first missionaries: this is a starting point for talking about developments in oenology over the centuries, describing the particular advances that have been made over recent decades and how consumer taste has gradually evolved.

## Further ideas

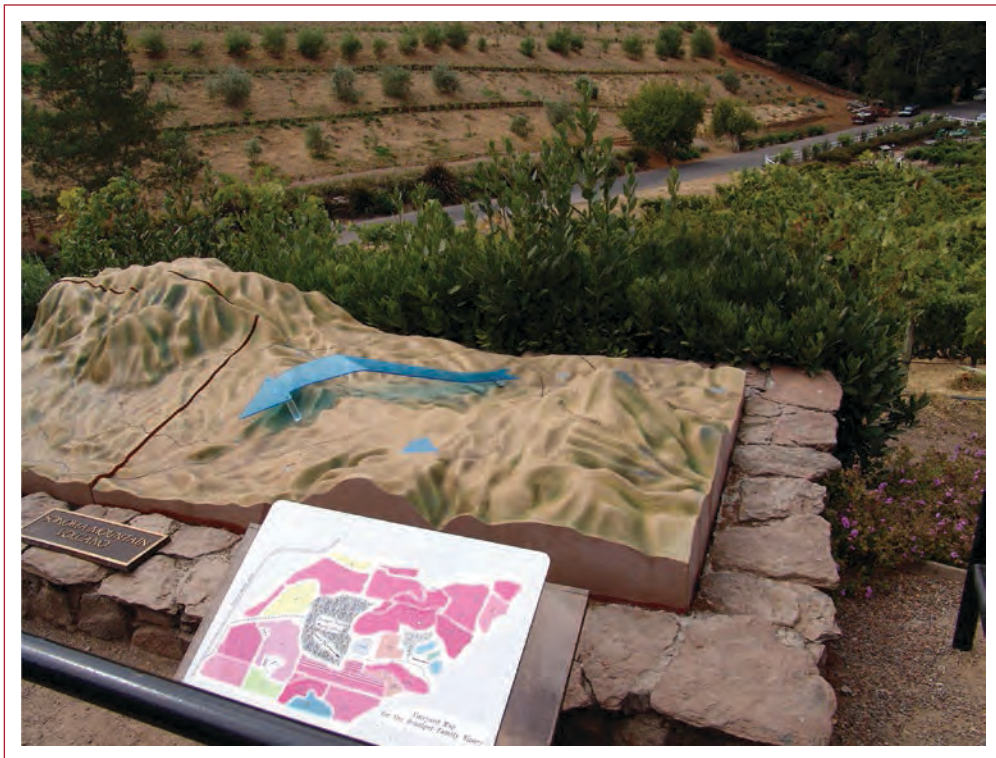
- Being able to visit the vineyards is an important feature on a tour for those who are seriously interested in discovering more about the estate's wines. Unfortunately this is not always possible, particularly if the vineyards are some distance away from where the wine is actually made. If the vineyards extend over a large area, it may be worth considering using motorized transport (e.g. a jeep or a minibus) to take small groups around the estate, with a driver/guide.
- The Benziger family winery (Glen Ellen, Sonoma, California) offers several tours a day at fixed times, for up to a maximum of 25 people. The tours are mainly for the mass market. The 'Biodynamic Vineyard Tram Tour' led by a driver/guide lasts 45 minutes and includes the chance to sample some of the estate's wines. The tram takes the visitors into the vineyard, with

a couple of stops along the way. The first stop, at a panoramic viewpoint, is dedicated to a description of the climatic conditions and geological features of the area, with the guide using a model to help to explain their impact on the soil and the terrain (Fig. 6.1). The second stop focuses on the winery's biodynamic approach to winegrowing and, if visitors are lucky, the chance to taste some grapes.

- The panorama is an essential part of a successful visit: the 360° 'panoramic guided tour' of the vineyards and nearby villages conducted by visitor guides from the Cantina Sociale Produttori del Barbaresco, in Piemonte, Italy, not only gives tour participants an exciting visual experience of the stunning landscape, but is also educational and informative. Visitors are

taken to the top of Barbaresco's ancient tower, originally built by the Romans and rebuilt in the 13th century, and from there they enjoy a visual tour of the countryside and learn more about the land laid out before them, one of the best and most productive wine regions in the whole of Italy. The sweeping view takes in the vineyards of Barbaresco and towards the south-west visitors can just get a glimpse of the town of Alba and the wine-growing area of Barolo. The plain and the rolling hills of Asti can be made out towards the north-east, while in the north-west, on the other side of the Tanaro River, the hills of Roero can be seen where the DOC wine Arneis is produced.

- Some wineries have created small botanical gardens where individual visitors can



**Fig. 6.1.** A geographical model in the vineyards to explain the physical characteristics of a regional *terroir* (Sonoma, California, USA).

take a varietal walk and discover different species of vines with the aid of an auto-guide. Clearly labelled flower and herb gardens also give tourists the chance to learn more about plant extracts and essences. This kind of experience is very useful, particularly if visitors later take part in a tasting session and undertake a sensory analysis of the wine.

### The wine production areas

- The group then enters the production area. At the entrance, a short video, with a live commentary by the guide, shows work going on in the vineyard, the cellar and the barrel ageing room.
- The visitors are led along a walkway between wooden fermentation vats. While the winery workers get on with their different tasks, the guide explains the company's philosophy towards winemaking, why they have made certain technological

innovations to their production processes and how the different machines work.

- The group then goes down to the level below, to where the bottoms of the wine vats are: there is a strong smell of must, the colour of the wine is studied and commented on. Visitors observe the steel vats and wooden barrels (Fig. 6.2). The guide is well prepared and is able to answer even the most technical questions and explain what each of the workers is doing, without ever disturbing them or interrupting their work.

### Further ideas

- Some wineries have foreseen the possibility of having visitors tour their production areas and have installed raised walkways in their cellars right from the outset, so that tourists can view the vinification tanks without disturbing the



**Fig. 6.2.** In the wine ageing room (Napa Valley, California, USA).

workers; this is the case, for example, at the Braida winery (Rocchetta Tanaro, Piemonte, Italy). Where this is not possible, it is always a good idea to advise tourists beforehand on the best way to enjoy their visit without compromising the working conditions of the winery.

- Penfolds, an Australian winery, has designed a 'Make Your Own Blend Tour', which is an excellent way to get visitors involved in the art of winemaking. After doing the classic tour of the winery, participants are invited into the Wine-makers' Laboratory to create their own personal blend of wine, using the same varieties of grapes that Penfolds uses for its wine production. At the end of the session, each person receives a customized bottle with their personal blend of wine as a souvenir of their visit.
- Other wineries have created rather unusual tours that combine viewing their wine production areas with brief 'lessons' in industrial archaeology. Maison Champy (Beaune, France), dating back to 1720, is the oldest *maison de vins* in Burgundy. Part of the building was designed by none other than Monsieur Eiffel himself. The tour includes a visit right to the top of the building where a machine, invented by Louis Pasteur to accelerate the maceration and fermentation processes of must, is housed. Pasteur's experimental ideas had to be abandoned in the end, but being able to imagine such a great scientist at work here provides a uniquely evocative experience for the visitor. Pietrantoni is a small winery operating in Abruzzo in Italy. It has two enormous underground cisterns that can hold up to 1402 hectolitres. Visitors are invited to step inside the cisterns, which are completely lined with Murano glass tiles.
- Some wineries may feel that they do not possess any outstanding features worthy of attention. However, a friendly, informative and professional approach

on the part of the guide and/or other winery staff may be more than enough to render a visit a memorable one. A visit to a tiny winery owned by Sylvie Spielmann, *propriétaire récoltant vigneron indépendant*, in Bergheim, Alsace, France, deserves a special mention. The excellent lesson on the meaning of *terroir* and the concept of *cru/grand cru* was supported by maps of the area showing the geological features of the vineyards. Having the opportunity to then taste and savour the wine from this mineral-rich terrain remains a lasting memory.

## Wine tasting

- The group then passes from the cellar to the tasting room. In the centre there is a large wooden table and enough chairs to seat everyone in the group comfortably. There are three glasses at each place.
- The visitors sit down and the guide hands each person three tasting sheets, which will be used to analyse three different wines. Bottles of mineral water are also placed on the table.
- As the first wine is being tasted, the guide outlines its characteristics. They then move on to the second tasting and then on to the third. The different members of the group are encouraged to use their olfactory and taste memories to identify the aromas and flavours of the different wines and to comment on their organoleptic characteristics. Even the most inexperienced visitors are invited to pass comment; the atmosphere is relaxed and friendly and not at all competitive, with the guide making sure that everyone feels involved. The first two tastings do not have any accompaniments (e.g. no bread or crackers on the table), but the third glass of wine is accompanied by delicious savoury *tartines* influenced by Californian fusion cuisine. The *tartines* provide an excellent introduction to food and wine



pairing; menu suggestions are made and advice given about which dishes would best accompany the Mondavi wines.

- The tasting session over, the guide thanks everyone for their attention and asks if anyone has any more questions. Visitors are invited to stay on should they wish; directions to the wine shop are given, as well as instructions for buying wine online and the advantages to be had by joining the Mondavi Wine Club.
- Just outside the tasting room, a lady is at work preparing *tartines* for the next group coming in, underlining the fact that each group is freshly catered for. A bowl of wine corks and wine labels (of the wines sampled in the tasting session) are placed on a side table; visitors can help themselves to these as they leave and take them home as souvenirs.

### Further ideas

- Food and wine pairing or matching is becoming ever more popular, with demand coming mainly from people who do not have much experience of the wine sector. Food and wine pairing sessions in the USA are enjoying enormous success, with some wineries basing their entire tour around this subject. There are many ways to integrate food and wine pairing sessions into a classic wine tour. At the Kendall Jackson Winery in Sonoma, California, tourists do not visit the areas dedicated to wine production. Instead, they are treated to sophisticated seasonal five-course wine and cheese or wine and chocolate menus, each course accompanied by a glass of Kendall Jackson wine under the guidance of a wine educator and enjoyed in the informal atmosphere of a wine bar. At the Peju Province Winery (Rutherford, Napa Valley, California), visitors do visit the cellars, but far more importance is given to tasting menus, culinary explorations and cooking classes that small groups can enjoy in the winery's own kitchens (Fig. 6.3). Here diners can observe the chef and the kitchen staff preparing the meal; the ingredients of the dishes are explained as they are served and suggestions made as to the best pairings.
- At Healdsburg, Sonoma, California, the Chalk Hill Estate 'Culinary Tour' includes a visit to the proprietor's private organic garden. The gardener, acting as guide, helps visitors to identify the different plants and essences. The formal food and wine pairing sessions have to be pre-booked. Groups (mainly couples or groups of friends) are catered for inside the elegant Estate Pavilion. An expert wine educator acts as host and an acclaimed chef is in charge of preparing the tasting menu. The Maison d'Olivier Leflaive (Puligny-Montrachet, Burgundy, France) offers a food and wine pairing programme entitled 'Table'. This consists of two fixed menus accompanied by many of the *maison* wines. An oenologist guides diners through the wines, while the proprietor pops in every now and then to see how the meal is progressing.
- In Riquewihr in the Alsace region of France, Jean Hugel (Hugel & Fils) created a tasting session that was almost an interactive game. Based on acquiring a few basic concepts, the aim of the session was to raise individual awareness about wine. The tasting was focused on four or five wines, beginning with a simple, everyday wine and ending with something more complex and prestigious. After the second wine was sampled, however, the normal rules for wine tasting were overturned and the first wine was tasted again. If the first wine was judged to be still pleasing to the palate, it meant that in terms of quality it was just as good as the second wine, in spite of its label and inferior price. The third wine was tasted and then, as before, attention focused on the first. If the latter still compared favourably, the same considerations were made, otherwise the third wine's superiority was agreed upon.



**Fig. 6.3.** Food and wine pairing menu in the winery's kitchen (Napa Valley, California, USA).

This approach continued until the last wine was tasted. In just over 10 minutes, participants learned the basics for being able to appreciate and distinguish simple wines from more complex ones of the same grape variety. The company has also published several brochures. One of these contains suggestions for pairing their aromatic wines (the quintessence of Alsace wine production) with oriental cuisine. Different sections are dedicated to the dishes of northern and southern China, Indonesian and Thai cooking, and Japanese and Indian food. The brochure also describes how to match the different qualities of individual wines (e.g. roundness, body, sweetness, freshness) to the flavours of the dishes (e.g. sweet and savoury or hot and spicy).

- It may happen that a visit to a winery is limited to paying for a glass of wine and sipping it at the bar of the wine shop. This usually happens when the winery's aim is to attract passing customers who are not particularly interested in knowing more about the product itself, but would perhaps like to stop and buy some bottles as a souvenir of their holiday in the area. The layout of the wine shop plays an important role in attracting these types of visitors, who appreciate being able to buy quality gadgets, wine accessories, design objects, etc. At the Imagery Estate Winery & Art Gallery (Sonoma Valley, California) the wine shop is set out like a permanent wine collection: the bottles are displayed under the same original paintings that are reproduced on the wine labels (Fig. 6.4).



**Fig. 6.4.** Wine and art at Imagery (Sonoma Valley, California, USA).

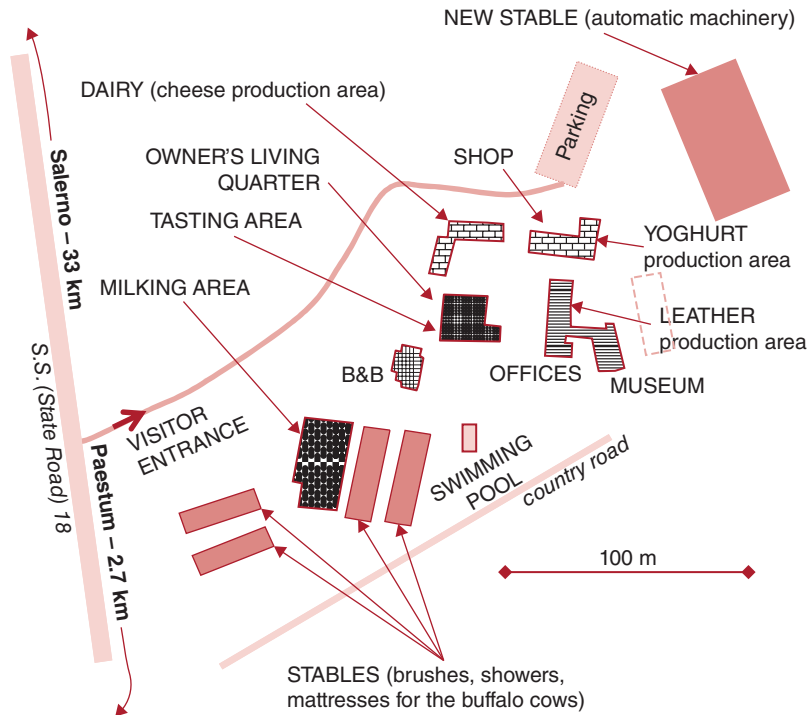
## AT THE DAIRY FARM

Dairy farms have the great advantage that they can offer visitors a complete overview of the production process, from the raw material to the finished product, in a short space of time. Being able to see fresh milk transformed into mozzarella and taste the still-warm cheese at the end of the tour is an undeniably gratifying experience.

### The visit

The Vannulo Dairy is located on the Palmieri family's organic farm and estate in Capaccio Scalo, Campania, Italy (Fig. 6.5). It has been chosen as a model for gastronomic tourism in

that it combines a high-quality product with well organized hospitality. An 18th century manor house on the estate has been completely restored and provides two comfortable suites for overnight guests. The farm, situated just a few kilometres from Paestum, a UNESCO World Heritage Site, is an organic water buffalo dairy farm. The mozzarella cheese produced here can only be bought from the estate shop and is not on sale anywhere else. The strong bond linking the land, the producer and the product is implicitly communicated and unconsciously assimilated by the visitor-customer. This is further reinforced when the visitors are taken down to see the buffalo herd. Visitors are stunned to see how the animals react when the tour guide calls individual buffalo by name.



**Fig. 6.5.** The Vannulo Estate Map. (From the authors' own map; the layout is based on the original map supplied by the Vannulo Estate.)

## Organization

- The tour must be booked well in advance and an entrance fee is charged.
- There are three tour options: farm and dairy tour, tour and light lunch, tour and full lunch. Lunch is served in the proprietor's private residence, dating back to the 18th century. Visitors who opt to have lunch are treated to a fixed menu and have the opportunity to sample some of the farm's products.
- The tour covers each phase of the production cycle.
- Groups are accepted (minimum number of participants: 10; maximum: 50).
- The tour lasts 75 minutes.
- Tours usually take place in the morning to allow groups to see the mozzarella cheese being made.
- The guide's approach is confident, friendly and informal.

## The tour

- The tour begins outside the cheese factory. A large window looking on to the production area allows the group to look inside and observe the cheese being made. The fact that visitors never enter the building itself means that working and hygienic conditions are never compromised. The guide explains each step of the production process: how the raw milk is first filtered and then coagulated, then the cutting of the curd and maturation (and the production of ricotta cheese from the residual serum); two people stretch and pull the obtained dairy product by hand and finally put it into brine to salt. Great attention is given to emphasizing the quality of the milk and to the fact that the cheese is made entirely by hand, resulting in a premium

product (Fig. 6.6). The guide also gives a brief account of how water buffalo first came to be introduced to the Campania region and the origins of mozzarella cheese, a fundamental ingredient of Neapolitan pizza that is actually a by-product of Provolone cheese.

- The tour then continues to see where the farm's yoghurt is made from pasteurized milk. The guide explains the functions and uses of the different machines, as well as giving a general description of all the farm's dairy products. The organoleptic characteristics of milk are discussed.
- The next stop is the leather handicrafts shop. The shop was set up to complete the supply chain of buffalo products. In reality, the skins are bought in from other suppliers.
- Visitors then move on to the farm museum, where examples of farm equipment and everyday utensils from different epochs are on display.
- After this, the visitors finally get to meet the buffalo. There are about 300 adult

female buffalo on the farm (although seemingly a large number, the quantity of mozzarella produced is relatively small). The group learns about buffalo behaviour and how the animals need to live in optimal conditions in order to produce milk of the highest quality (they are even given homeopathic treatments when necessary). Visitors watch while some of the buffalo cows pass under the showers and have their coats brushed. The buffalo are then allowed to relax on special mattresses in their stalls. A new stable has recently been installed for automatic milking. The guide gives more information about organic produce and the sustainable farming methods practised by the farm's owners. The expanse of estate land guarantees that there is a correct balance between the space given over to the buffalo herd and the area utilized for cultivating forage to feed the animals (each buffalo cow is given at least 25 kg of grass a day). The most exciting moment comes



**Fig. 6.6.** A plump buffalo mozzarella (Paestum, Campania, Italy).

when those who wish to do so are invited to stroke one of the animals. Being able to look a buffalo in the eye and come up against such docile grandeur is a truly unforgettable experience.

- In the estate shop, visitors stand and sample fresh mozzarella balls. The shop also sells yoghurt, ice cream and cappuccino, all made from the farm's own buffalo milk. Finally, the guide ends the tour by explaining the distinctive sensory characteristics of the product.

### Further ideas

- The cooperative members of The Valtellina Dairy Group (Latteria Sociale Valtellina) in Delebio (Italy) offer the opportunity to discover the local alpine cheese production. Tourists can hike up to the mountain pastures, watch the cows being milked and sample Bitto cheese (a PDO cheese, exclusive to mountain areas and only produced in the summer months). Accommodation can be arranged should they wish to stay overnight in the high mountains.
- The Cooperative of Milk and Fontina Cheese Producers in Valle d'Aosta (Italy) runs a Visitor Centre in Valpelline. In order to give visitors a complete picture of how Fontina is made, the centre has been divided into three sections, focusing on the history, the *terroir* and the methods used to transform milk into the finished product. There is no strict viewing order and visitors can move from one section to another as they please, watch a video, or visit the small museum and photo gallery housed inside the centre. In food tourism, however, the senses are all-important, so the cooperative has organized guided tours of the cheese maturing room, located beside the Visitor Centre. An enormous space that can hold up to 60,000 cheeses at any one time, the maturing room was once the entrance tunnel to an old copper mine, and even today the railway tracks that were once used to transport copper are now used to move the cheese. The space is cold and damp, impregnated with an overwhelming smell of ammonia. Here, visitors learn how each cheese is individually stored and handled (in extremely difficult working conditions) until it reaches the correct stage of maturation (Fig. 6.7). The tour ends with a tasting of Fontina cheese.
- Cheese tasting courses give gastronomic tourists the chance to discover and compare the characteristics of cheeses from different production areas and producers, at different stages of maturation or at different times of year. These short courses can be organized by individual *affineurs* (cheese agers) in their cheese ageing rooms or sales points. It can be very stimulating to participate in a cheese tasting session in an unusual and evocative location such as an old community dairy: a memorable experience for the visitor.
- The Fromagerie Gaugry (Gevrey-Chambertin, Burgundy, France) receives raw milk from about 30 dairy farmers in the area. The milk is used to make seven different types of unpasteurized soft cheeses with a washed rind, including the renowned AOC L'Époisse. The cheeses need to mature in cold, damp conditions and the original buildings were so cold and inhospitable that only small numbers of people could be accommodated for a very short space of time. The dairy has now constructed new buildings on the premises that allow greater numbers of visitors to view the whole production cycle comfortably (Fig. 6.8). Both individual visitors and groups are catered for. Independent tourists and guided groups follow the same route, along a walkway that runs parallel to the different production rooms, and they can observe all the different phases of production through large transparent glass panes. Information panels and a simple leaflet printed by the company (which must be handed



**Fig. 6.7.** Tools for brushing Fontina cheese (Val d'Aosta, Italy).

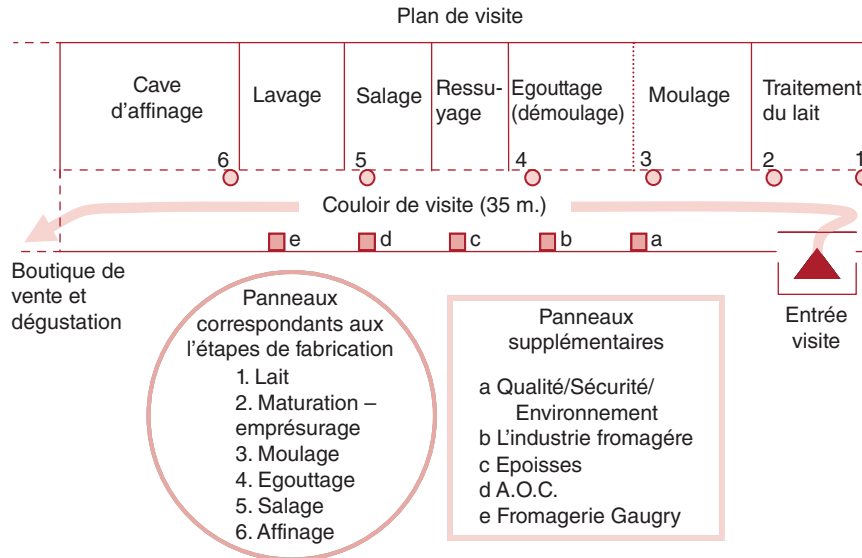
back at the end of the visit) help to guide the individual visitor, while those on the guided tour are treated to a tasting of the company's products accompanied by a glass of Burgundy wine.

## AT THE BREWERY

Breweries always have great appeal, whether they are small artisan breweries or large beer factories. Particularly in countries where it is the preferred drink to accompany a meal, beer lovers and connoisseurs get great pleasure from discovering new brews. There is something very satisfying about sipping a beer in congenial surroundings, served at just the right temperature, in just the right glass.

### The tour

- The family-run Cantillon Brewery situated in the heart of Brussels (Belgium) has been making beer for over 100 years. Lovers of Lambic beer flock here to enjoy a drink in surroundings that have not changed since the brewery opened in 1900. Since 1978, it has been a 'living museum' informing visitors about the history of the brewery, their methods of production and the difficulties involved in producing a naturally fermented beer in such a particular microclimate. Opening their doors to visitors was the only way the family could keep the brewery in the public eye and their traditional production methods alive in a rapidly changing market. The tour naturally



**Fig. 6.8.** Tour organization of the production areas of Fromagerie Gaugry. (From the authors' own map; the layout is based on the original map supplied by the Fromagerie Gaugry, Bourgogne, France.)

focuses on the artisan methods used to make the unique-tasting Lambic beer. Individual visitors are given a simple A4 sheet that guides them around the dimly lit production areas that have remained almost unchanged since the founding of the brewery. Groups of visitors, on the other hand, are given a guided tour and can watch the beer being made during 'public brewing sessions'. The tour does not follow the different phases of the production cycle in chronological sequence (clearly illustrated in the leaflet), but takes visitors through a number of production areas located on different floors of the building. The visit begins with the area dedicated to the brewing process before moving on to view the hot water tanks and the mashing and grinding room. The cereals (wheat and malted barley) used to make the beer are stored and displayed in the attic, along with the hops that are used to give the beer flavour. In the chilling room there is an enormous red copper container where

the must is cooled by air filtering through the Persian blinds. This cooling process takes place from the end of October to the beginning of April. As the blinds are opened and closed to create a draught, the air carries the wild yeast spores that set off the process of natural fermentation. It is here that the visitors begin to understand how the room's microclimate and microorganic balance create the right conditions to produce a spontaneously fermented beer. After this, the group is taken to the barrel room to view the fermentation and maturation phases, to see the bottles being washed and the beer being bottled. They may also have a chance to see how Gueuze or beers with fruit are made. A visit to the beer cellar is completed by sampling the brewery's products (Gueuze, Kriek, etc.).

- Just a short walk from the centre of Dublin and located in the heart of the St James's Gate Brewery, the Guinness Storehouse is Ireland's number one tourist attraction. Guinness is also one



of the world's top brands (every year the brewery manufactures millions of litres of its renowned stout). The Storehouse, with over 100 employees, is also very impressive from an architectural point of view. It was originally built at the turn of the 20th century in a style inspired by the Chicago school of architecture; it is an amazing structure supported by massive steel beams. The building was renovated between 1997 and 2000 and now covers an area of almost 16,000 m<sup>2</sup> distributed over seven floors. In the centre there is an enormous glass atrium shaped like a huge pint beer glass. On first impressions, the Guinness Storehouse tour would seem to be constructed according to the criteria of 'post-modern tourism'; actually, the tour's approach is deeply rooted in Dublin's history, culture and traditions. Visitors are not taken on a tour of the production areas. The tour concentrates instead on communicating some very clear messages, including the company's contribution to Dublin's economy, history and culture and the importance of responsible drinking. The guided tours, available in four foreign languages (French, German, Spanish and Italian), are for pre-booked groups only. Individual visitors can take a self-guided tour covering all seven floors, organized into different thematic sections with hands-on activities, interpretive panels, videos, interactive displays and so on. The different sections are divided into: How Guinness is Made (history, production methods, ingredients, art and passion); Global Dimensions (markets and transportation); The Story of Arthur Guinness (founder); 'My Goodness, My Gilroy' (75 years of advertising); The History of a Building (the story of the building's transformation from fermentation plant to top tourist attraction); and finally, on the seventh floor, the Gravity Bar. On showing their entrance ticket (in fact a souvenir drop of Guinness in

a small plastic pebble), visitors receive a complimentary pint of Guinness and enjoy a 360° panoramic view of Dublin city. The first stop, halfway through the tour, is designed to give visitors the rudimentary basics of beer tasting techniques and to explain the best conditions for drinking beer, with tastings of Guinness, Guinness Extra Stout and Foreign Extra Stout. Under-age visitors are served a soft drink. The Storehouse also has an independently run retail shop on the ground floor. Corporate clients are well catered for and designated spaces can be hired for meetings, conferences, workshops and training courses. The fact that the tour essentially attracts mass tourism means that there is a risk it could be just another 'theme park' experience. However, the visit is so well organized and has been planned so carefully that it never falls into the trap of becoming banal or unoriginal. The strong link between the product and its city of origin is continuously emphasized throughout the tour; this is reinforced by the fact that the building itself is part of the city's urban landscape. With the aid of a specially produced panoramic guide on the window giving the names of Dublin's most important buildings, visitors can easily identify the city's most significant landmarks from the Gravity Bar on the seventh floor (Fig. 6.9). The names of the landmarks and quotations from Dublin's authors are etched on the glass windows.

## AT THE OLIVE MILL

In spite of the fact that olive oil consumption is part of the Mediterranean tradition, the produce's organoleptic and sensory characteristics are relatively unknown to the general public. This is even truer for foreign visitors where olive oil is not part of their culture.



**Fig. 6.9.** Enjoying the urban landscape while drinking a pint of stout (Dublin, Ireland).

A visit to an olive mill, therefore, can be an exciting revelation for both domestic and international tourists. Here they can discover what essential characteristics an oil must possess in order to qualify as an extra-virgin olive oil, the importance of timing and harvesting methods, how olives are cultivated, the kind of results obtained from continuous or discontinuous pressing cycles, stone grinders, hammer presses or state-of-the-art olive presses incorporating the latest technology. The sensory characteristics of olive oil change according to where it is cultivated and visitors can discover the defining notes of an olive oil from one mill and then go on to compare it with other locally produced olive oils.

Most people nowadays are aware of the health benefits of a Mediterranean diet, so they will be interested to find out more about the nutritional properties of olive oil and the best ways to conserve the product. If a tour

takes place outside production time, the guide must make every effort to involve the visitor and try to give a clear and lively picture of the atmosphere and frenetic activity of life on an olive farm during the harvest.

Olive oil tasting sessions tend not to be technical, unless of course visitors already have some experience or are professionals in the field. Visitors generally sample the oil as it is used in local cuisine, e.g. poured on crusty bread.

Another point in favour for those who may be considering opening their doors to tourism is the remarkable variety of landscapes that are a feature of olive growing areas. In Spain, for example, there are the olive groves and the picturesque whitewashed houses of the *pueblos blancos* of Andalusia where olive oil is often processed by large cooperative olive mills. In Italy, the geomorphological features of the land are so varied that olives are grown in an array of stunning landscapes: the terraced

olive groves of Liguria; the centuries-old olive trees of Puglia; the olive ‘woods’ of Aspromonte; the new regimented olive groves in the Sibari area (Calabria); the mixed-cultivation fields (olive trees and cereals) of the Marche; the contrast of the hills in the PDO areas of Abruzzo with the surrounding steep slopes of the Maiella and Gran Sasso mountains; the calm waters of Lake Garda with its olive groves and the alpine foothills; the rolling hills of Umbria and Tuscany dotted with olive trees that were the inspiration of so many Renaissance painters; and so on.

### The tour

At the Galantino Olive Mill in Bisceglie (Puglia, Italy), the retail shop, on two floors, sells a wide range of the farm’s products, from bottles of extra-virgin olive oil to a selection of olive oil speciality products and oil-based cosmetics. Also inside is a meeting point for visitors, a small conference room and a

dedicated space for olive oil tasting. The guided tours around the farm’s 18th century olive mill where the olive oil is made are free. Already engaged on the farm in a variety of roles, the guides are familiar with the company’s business philosophy and strategies as well as being specifically trained to welcome and guide visitors. The tour begins with an explanation of the functions of the machines inside the mill, including ancient granite millstones (Fig. 6.10). The characteristics of the terrain are described along with sustainable and organic farming methods, what happens during the olive harvest, the variety of olive plants, how the olives are picked and cleaned, storage, what happens when the olives are crushed into a paste and then sent to the extractor to remove water and other residues, bottling, the sensory characteristics of the product and suggestions for using olive oil in cooking. The guide spends some time explaining the *terroir* and describing the characteristics of Coratina and Ogliarola, olive cultivars that are native to this particular area north of Bari. The



**Fig. 6.10.** A working grindstone olive mill.

extra-virgin olive oil obtained from these varieties has a distinctive spicy and bitter taste. The guide then goes on to recount the history of the Galantino Olive Mill that goes back 100 years. Each step in the development of the mill is retold, together with accounts of the people who were responsible for initiating change and progress: the purchase of the mill, the purchase of the land, the relationship with local farmers who still bring an additional quantity of their olives to the mill to be pressed, the prizes won and the achievement of international recognition. The guide then describes how there is increasing demand for high-quality olive oils with distinctive sensory qualities. The mill has had to respond to these changes in consumer behaviour by producing a wider range of products to suit more sophisticated tastes. The tour ends with a free tasting of some of the mill's products. Visitors can choose which products they would like to sample, ranging from traditional extra-virgin olive oil to organic, fruit- or herb-infused olive oils. The mill also receives large groups of tourists. They are offered tasting workshops in the showroom, as it can accommodate a greater number of visitors. Another interesting point for visitors is to learn that different varieties of extra-virgin olive oil can be listed according to their *terroir* and sensory characteristics (similar in a way to a wine list), so that restaurateurs and gastronomes can choose which oil would best accompany and complement dishes on a menu.

### Further ideas

- Some olive oil companies have parallel production lines (often working simultaneously): the continuous pressing method, which is the most modern, running alongside the more traditional or discontinuous method, or two to three phased production processes. In this case, the tour experience is even more exciting as visitors get the chance to sample the end products and compare the differences between them.
- Tourists or professionals who already have some knowledge about extra-virgin olive oil production are usually fascinated by the changes that new technologies have brought to the sector. The Monteschiano Olive Mill in Maiolati Spontini (Marche, Italy) is an excellent example of a company that has invested in cutting-edge technology for olive oil extraction and machines designed by the Pieralisi Group (who are also proprietors of the mill). Visitors to their website ([www.frantoiomonteschiano.com](http://www.frantoiomonteschiano.com)) could watch the different phases of olive oil production on live webcam.
- Olive oil tasting sessions are much more satisfying and suggestive when they take place in buildings or spaces that have a characteristic style and architecture. In Puglia, for example, one of the largest olive growing regions in the world, tastings can be held in some of the underground olive mills of Salento. The mills were traditionally built underground for safety reasons and to keep temperatures at just the right level for optimal production and storage. Also in Puglia, olive oil tastings are organized in the legendary *trulli* of Valle d'Itria. Short courses for those wanting to experience being on a tasting panel can also be organized on request.
- The area of Ferrandina (Basilicata, Italy), where the Lacertosa Olive Mill is located, is famous for a particular variety of olive called Maiatica. At the mill, visitors not only watch oil being extracted from the olive drupes but are also invited to see the rather complex but traditional local custom of baking the Maiatica olives. These are delicious tasted on their own or are used as an ingredient in a number of local recipes.
- Diners at the Archibusacci Mill Restaurant in Canino (Lazio, Italy) can look out through a large window and see one of the olive mills while enjoying *bruschetta* (extra-virgin olive oil poured on to toasted bread) and other local dishes using the

mill's own olive oil. A similar experience is reserved for tourists at the Fratelli Carli Olive Oil Museum in Imperia (Liguria, Italy). The vast production area is protected by an enormous glass panel so that visitors can observe what is going on as they walk past on their way to the museum.

- There is increasing interest in olive oil products, particularly in the health, beauty and leisure sectors. Many rural farms are combining their production activities with tourism hospitality, offering guest accommodation and on-site beauty centres, using their own olive-based cosmetic products and oils.

## AT THE DISTILLERY

A guide in charge of taking visitors around a distillery needs to be able to explain the different phases that go into producing liquor or spirit and the function of a still. Visitors also need to know that distilled beverages are the result of fermented raw materials such as grapes, malt, fruit and herbs.

### The visit

'The Jameson Experience' at the old Middleton Single Distillery in County Cork, Ireland, takes place inside the original industrial plant dating back to the 17th century. Unlike other big brand names in the whisky business, not all Jameson's tours include a visit to production areas. Instead, a smart guide in uniform, at fixed times throughout the day, may accompany visitors on a tour that is almost a 'history lesson' in industrial archaeology. The scope of the tour, however, is broad enough to captivate everyone's interest. It begins with a video about the production of Jameson whiskey, the main theme of which focuses on the history of the distillery, the buildings and factory life as it was in the past. Inside the old distillery the machines are no longer in use but they serve as an eloquent testimonial of production methods hundreds of years

ago. The guide explains the similarities and differences in whisky production today, and the methods the company uses for storage and distribution. A great deal of emphasis is placed on explaining the differences between Irish whiskey and scotch. Scotch whisky is made by first drying the barley malt over fires that have been fuelled with dried peat. The result is a distinctive smoky flavour, quite different from Irish whiskey. Another difference is that most Irish whiskey is distilled three times. At the end of the tour, visitors can enjoy a glass of whiskey at the company bar (included in the price of the ticket). The old distillery also houses a restaurant that offers traditional Irish dishes and home-made cakes. The on-site shop is the only place in the world that sells bottles of Jameson's 'Reserve'. Customers can buy their own personalized bottle of 'Reserve' and have their name printed on the label. In spite of the fact that the tour is repeated over and over again during the day, the guide still manages to be friendly, communicative and engaging. The highlight of the tour is when the guide asks for three volunteers to come forward to take part in a whiskey-tasting workshop. The volunteers sit down at one of the tables in the bar and they are given five different whiskeys to taste. Three of them are Jameson's whiskeys, one is a scotch and one is a bourbon. The glasses are placed on a paper tablecloth specifically designed for the tasting workshop. The guide asks them for their impressions and sensations after each tasting, while also explaining the basic techniques for tasting spirits and how the sensory notes of the different drinks should help the tasters to identify them (Fig. 6.11). At the end of the workshop each of the volunteers receives a tasting certificate. It is an enjoyable end to the tour, particularly for the three 'tasters', who feel that they have had a privileged experience compared with their tour companions who were there only as observers. However, even those who did not actually take an active part in the workshop come away entertained and much more informed about tasting techniques.



**Fig. 6.11.** Comparing whiskeys, tasting session (Midleton, Ireland).

### *Further ideas*

- ‘Bolle’ (literally ‘bubbles’) is the name of the futuristic structure designed by Italian architect Massimiliano Fuksas for the Nardini Distillery in Bassano del Grappa (Veneto, Italy). It can accommodate groups of eight to ten people up to a maximum number of 50, on request. The tour lasts about an hour and includes a visit inside the auditorium, a short video and a grappa tasting workshop held inside the company’s brand new shop. The Nardini family has been producing grappa since the 18th century and the quality of its products is renowned. However, many tourists come here not just because of the Nardini name; they

come because of their interest in contemporary architecture and the work of Fuksas, who is probably one of the best-known living Italian architects. His structure is intended to represent visually the process of distillation and the transformation of the raw material. Two transparent ellipsoid bubbles hang suspended between the trees, symbolizing the mercurial lightness of alcohol (the still and the beverage) and the tenaciousness of the earth (the grapevine rooted in the soil). The bubbles actually house the company’s research laboratories, while the underground auditorium has been added to host events and receive visitors. There is a small pool of water at ground level.

- For those who couldn't make the journey to Lynchburg, Tennessee (USA) to tour the Jack Daniel Distillery, the company website ([www.jackdaniels.com](http://www.jackdaniels.com)) used to provide detailed information about the original founders, its history, location, products and production as well as ideas for recipes and the possibility to take a virtual tour. To make the tour more realistic and to give virtual visitors a greater sense of participation, they could choose from one of the distillery tour guides available on the site. At the end of the tour they could sign their name in the virtual visitors' book, express an opinion about the tour and respond to company questions devised to pick up on consumer behaviour patterns and tastes. They were also asked to leave their address, a useful marketing strategy for expanding the company's mailing list and keeping in contact with future customers. The online virtual tour is no longer available, but the distillery offers some themed tours. Those who have been lucky enough to visit the Jack Daniel Distillery in person can download a photo of themselves with their tour group by clicking on 'Photo Pick Up' on the home page and typing in the date of their visit.
  - In Scotland, land of whisky, the 3-mile 'Three Distilleries Pathway' in Port Ellen (Islay) is fully accessible for walkers, cyclists, pushchairs and wheelchairs. It reflects on a bigger scale the Speyside Malt Whisky Trail, allowing different target groups ('active', 'creative', 'outdoorsy', 'foodie', 'connoisseur', 'craftsman', 'in a hurry', 'staying a while', 'looking for events') to discover factories and the best of surrounding landscapes ([www.maltwhiskytrail.com](http://www.maltwhiskytrail.com)).
- mills and distilleries. Virtually every single food or wine resource has the potential to stimulate the consumer's senses. Being able to view the whole process of production, from raw material to finished product, is bound to excite visitors' curiosity, particularly when they are able to feel the presence and taste the flavour of a region through its products. Any food and wine resource, therefore, has the power to become a resource for tourism. Other food production centres that have opened their doors to tourism include the following.
- The rice fields and farmhouses of northern Italy, where visitors learn about rice cultivation in the past and in the present, the different varieties and their characteristics, how the end product is obtained and its different uses in cooking. Tours generally start with a visit to the rice field and end with sampling a traditional risotto, or for those fortunate enough, a tasting menu entirely based on rice, from the hors-d'oeuvres to the dessert (Piemonte, Lombardia, Veneto).
  - Artisan bread makers, e.g. producers of Altamura bread in Puglia, *coppia* bread in Ferrara (Emilia Romagna) and Sardinian *carasau* bread, but also factories providing basic bread making courses for non-experts, among which children can experience hands-on activities to share with their peers while learning basic information on health and nutrition (e.g. Haubi, in Austria).
  - Cured meat factories. There are many renowned ham producing companies in Spain and Italy who welcome visitors into their ageing cellars, explain the process of ageing and offer samples of the different cuts. There are also the smaller artisan producers operating in distinct regions in Italy, such as the Tuscan farmers who produce salami and prosciutto from the prized Cinta Senese pig, or spicy 'Ventricina' in the southern part of Abruzzo.
  - Producers of traditional balsamic vinegar in Modena and Reggio Emilia (Italy).

## OTHER PRODUCTS, OTHER VISITS

Food and wine tourism is not just about visiting wineries, dairy farms, breweries, olive

- Producers of *foie gras*. Tours include being able to see where the ducks are bred in semi-freedom and a demonstration of *gavage* (force feeding); tours end with informal product tastings or even a sit-down meal. In France, producers often organize culinary tours, gastronomic weekends and cooking courses.
- Professional figures involved in the salmon supply chain: fishermen, salmon smoking factories, salmon retailers, etc. (Fig. 6.12). Tours are sometimes conducted by marine biologists. In Ireland, week-long gastronomy tours are organized during which guests follow the entire cycle of the fish's life. Lessons are practical as well as theoretical: participants learn about smoking techniques and the art of wine matching and enjoy tasting workshops.
- Pasta factories, where visitors can see pasta being made and enjoy a meal of hot pasta at the end of the tour (Italy).
- Snail farms, where the whole life cycle of a snail can be followed (Belgium).
- Salt works. For example in Italy in Marsala and Paceco (Sicily), Margherita di Savoia (Puglia) and Cervia (Emilia Romagna), visitors are taken on a tour of the salt route and learn about industrial heritage, modern methods, and even windmills that were used in the early processes of salt making, ending the visit with tasting different kinds of salt.
- Fruit production centres, where apples are delivered direct by the farmers to be selected, washed, packaged and stored. A tour of this type could begin in the apple orchards, follow parts of the automated production line and end with tasting the



**Fig. 6.12.** A *tonnara* (tuna fish processing plant): industrial archaeology in Sant'Antioco (Sardinia, Italy).



various types of product produced at the centre, e.g. fresh, dried or puréed apples.

- Artisan chocolate makers (Brussels, etc.), where chocolate is still made according to age-old recipes. In Modica, Sicily, the tradition of making chocolate with *peperoncino* (chilli pepper) may have come from the Spanish, who learned the art of chocolate making from the Aztecs; in the same area, chocolate is used as an ingredient for sweets filled with meat.
- Cocoa plantations, where tour visitors are taught the necessary skills to be able to appreciate the sensory characteristics of a *grand cru* chocolate and are taken through the different stages of transforming cocoa into chocolate, learn about and look at the traditional ways and methods used by the local people to prepare chocolate, and sample the products in a final tasting session; sometimes this can be complemented by chocolate-based beauty treatments (Mexico, Belize, etc.).
- Tea plantations, where tourists can learn how to pick the leaves during the harvesting season, see how the raw leaves are fashioned into tea and, while enjoying the beverage at the end of the tour, learn the ancient art of making tea (China).

There are many other activities and initiatives that fall under the umbrella of food and wine tourism:

- Fishing tourism. Offering tourists something more than just a seaside holiday, this gives visitors the chance to experience a destination's underlying culture and to learn about responsible fishing practices as opposed to the unsustainable methods of the mass fishing industry. A tour could include going out in a fishing boat with local fishermen, learning how to catch fish with them and how to prepare the fish ready to eat either on board or back on land. Some fishing tours also offer overnight accommodation with a fisherman's family.
- Farmers' markets. Visitors can have guided tours and meetings with the food producers.
- Farm days and stays. Joining in everyday farm activities such as fruit picking, helping with the grape harvest, milking the cows, etc., working guests usually get the chance to sample farm products, which more often than not they purchase to take home with them (e.g. bottles of wine, extra-virgin olive oil, jam).
- Model farms. These give young visitors the chance to learn all about work on a farm. Particularly important for inner-city children who have no experience of rural life, model farms generally cater for school groups. Even the youngest visitors can join in with different activities such as sowing and harvesting, and learn how raw materials are transformed before they arrive in supermarkets or appear on their table at home. Having direct contact with farm workers and farm animals and seeing how crops are grown and picked awakens city children's senses and helps to raise awareness about the importance of farming and the rural economy and the need to safeguard natural environments.
- Cookery schools and courses. Working alongside a chef, participants learn about regional products or those of a particular producer, develop their prowess in the kitchen and sit down at the end of the course to enjoy the meal that they have prepared during the lesson. They go home armed with new recipes and new skills.
- Sustainable and responsible tourism projects. With the aim of stimulating production and local economies in developing countries also through tourism, an example of this type of project is a tour of a coffee plantation, where visitors learn all about the cycle of coffee production from plant to bean selection to cup. They can also learn about the local coffee culture by participating in a traditional coffee

ceremony in local people's houses. Coffee is also offered in specially designed visitor centres, e.g. in Ethiopia. (In a completely different context, 'coffee crawls' to visit coffee houses in Seattle are based on first-hand knowledge of coffee brewing and tasting.)

- Monasteries. Apart from enjoying a historical or cultural visit, tourists can buy produce/products such as honey, fresh fruit or liqueurs, cultivated or made by the monks.
- Food-themed walking tours in urban environments during which the participants stop more than once along the route to taste local products and/or recipes and/or beverages (e.g. pub crawling in London and Dublin, street food worldwide).
- Visits to food and wine museums in places renowned for agro-food production (but considering also the knowledge of today's real local economy based on food).
- Environmental awareness programmes. These include initiatives to safeguard rural and mountain environments and their traditional activities, e.g. supporting alpine dairy farming. It is possible for a city dweller to adopt an animal and contribute to the expenses involved in breeding and looking after them. In exchange, they receive dairy products made from their 'own' herd. The cooperative ASCA in Anversa degli Abruzzi (Italy) has come up with a campaign called 'Adopt a Sheep, Defend Nature'. The people who adhere to

this initiative can have lamb, milk, cheese, wool and fertilizer, and are welcome to stay on the farms that are members of the cooperative. In this way, they can participate directly in the working life of the farm and appreciate from close quarters what sheep breeding actually involves (grazing, birth, feeding, shearing, transhumance). In Valsugana (Trentino, Italy), people can adopt a cow, choosing the one they like best. In summer the adopter can go and visit 'their' animal grazing on the meadows of the Alps and get their own cheese made in a *malga* (Alpine milking station) ([www.visitvalsugana.it](http://www.visitvalsugana.it)). To help small farmers, there are tree adoption programmes as well: from California to Australia the adopters are asked to pay an annual sum of money for having their own personal peach or apple tree looked after by the farmers and are given the chance to harvest their own fruit themselves, in flexible dates during the right season.

## REVIEW QUESTIONS AND TASKS

- Wineries, dairies, breweries, olive mills, distilleries and others: write in a few lines the welcome speech you would give before beginning the tour. Illustrate the itinerary, places where you'll be stopping and activities.



# Cherries

BOTANY, PRODUCTION AND USES

Edited by José Quero-García, Amy Iezzoni,  
Joanna Puławska and Gregory Lang

 **cost**  
EUROPEAN COOPERATION  
IN SCIENCE & TECHNOLOGY

 **CABI**

# 17 Fruit Chemistry, Nutritional Benefits and Social Aspects of Cherries

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## 17.1 Introduction

Cherry nutritional composition, phytochemical content and antioxidant capacity should be considered on a cultivar/genotype basis since many new cultivars that enter into the market through the breeding programmes (Sansavini and Lugli, 2008) have apparent differences for both qualitative and phytochemical antioxidants contents (Ballistreri *et al.*, 2013; Goulas *et al.*, 2015). The breeding programmes have led to the release of numerous cultivars, where the main attributes considered were bearing habits, ripening period, fruit size and yield, increased fertility, reduced susceptibility to environmental damage and diseases, extension of seasonality, especially for early-ripening genotypes, and resistance to cracking. However, to the best of our knowledge, phytochemical status and nutritional properties are not being evaluated through the breeding programmes.

This chapter focuses on the physicochemical characteristics (soluble solids, pH, titratable acidity, volatile compounds) and nutritional (e.g. carbohydrates, proteins,

lipids, sugars, organic acids, minerals, vitamins) and non-nutritional (other constituents with biological properties beyond nutrition) composition of sweet and sour cherry fruits. Non-nutrient food constituents include phytochemicals, phytonutrients, plant secondary metabolites, and bioactive and health-promoting compounds.

## 17.2 Fruit Chemistry

The chemical characteristics of sweet (*Prunus avium* L.) and sour cherries (*Prunus cerasus* L.) have been widely reviewed, not only because they largely affect the sensory quality of the fruit, but also because they have a strong influence on consumer acceptance (Crisosto *et al.*, 2003). Furthermore, physicochemical studies are also relevant for producers for proper design of the harvesting and postharvest technology for sweet cherry production in the world (Hayaloglu and Demir, 2015). The large and diverse reported values of pomological characteristics of cherries denote how these

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properties are highly influenced not only by the cultivar, but also by other environmental variables, such as climatological conditions and geographical origin (Faniadis *et al.*, 2010; Tomás-Barberán *et al.*, 2013).

Both sweet and sour cherries present a very low caloric content: 63.0 kcal (263.34 kJ) per 100 g for sweet cherry and 50.0 kcal (209 kJ) per 100 g for sour cherry (USDA ARS, 2016). They are also considered an excellent source of numerous nutrients and phytochemicals (McCune *et al.*, 2011), which is one of the major reasons for their increasing popularity in the human diet. In addition, many epidemiological studies have established that their regular consumption is associated with health benefits and the well-being of individuals (Ferretti *et al.*, 2010; McCune *et al.*, 2011).

### 17.2.1 Total soluble solids

The value of total soluble solids (TSS) in sour and sweet cherries may reach up to 24.5 g per 100 g fresh weight (FW) (Table 17.1). This parameter is an important factor in determining the consumer's acceptability (Crisosto *et al.*, 2003; Valero and Serrano, 2010). For sweet cherry, reported values range from

as low as 12.3 g per 100 g FW in 'Van' (González-Gómez *et al.*, 2010) to 24.5 g per 100 g FW in 'Salmo' (Girard and Kopp, 1998). Such differences in TSS can be attributed to microclimatic conditions, rootstock selection and planting system, as well as differences in the physiological stage adopted as a harvesting criterion (Goulas *et al.*, 2015). Finally, it has also been reported that TSS must be above the threshold of 14.0–16.0 g per 100 g FW as acceptable for marketing cherries (Crisosto *et al.*, 2003).

In the case of sour cherry, the average values found in commercial cultivars are around 15.0 g per 100 g FW, while only a few cultivars are above the threshold of 17.0 g per 100 g FW (Grafe and Schuster, 2014). Interestingly, autochthonous sour cherry genotypes in Portugal were found to have TSS values in the range of 17.4–22.8 g per 100 g FW (Rodrigues *et al.*, 2008). Hungarian sour cherry cultivars also showed appreciably higher levels of TSS (up to 23.1 g per 100 g FW in cultivar 'Pipacs 1') (Papp *et al.*, 2010).

### 17.2.2 Titratable acidity

Titrateable acidity (TA) is one of the most important attributes in cherry, since it is also

**Table 17.1.** Standard physicochemical attributes of sweet and sour cherries.

Species	TSS (mg per 100 g FW)	References	TA (g malic acid per 100 g FW)	References	Maturation index (TSS/TA)	References
Sweet cherry ( <i>Prunus avium</i> L.)	12.3–24.5	Girard and Kopp (1998); González-Gómez <i>et al.</i> (2010)	0.7–1.2	Serradilla <i>et al.</i> (2016)	8.6–24.4	Usenik <i>et al.</i> (2010); Serradilla <i>et al.</i> (2012)
Sour cherry ( <i>Prunus cerasus</i> L.)	15–23.1	Papp <i>et al.</i> (2010); Grafe and Schuster (2014)	1.3–3.1	Rodrigues <i>et al.</i> (2008); Papp <i>et al.</i> (2010); Rakonjac <i>et al.</i> (2010); Damar and Ekşi (2012); Grafe and Schuster (2014)	5.8–15.8	Wojdyło <i>et al.</i> (2014)

FW, fresh weight; TSS, total soluble solids; TA, titrateable acidity.

directly related to the acceptability by consumers, and it is a highly cultivar-dependent parameter. Sweet cherries are considered as mildly acidic fruits with pH values between 3.7 and 4.2, while sour cherries range from pH 3.1 to 3.6 (Serradilla *et al.*, 2016). Regarding TA, important differences have been observed between sweet and sour cherry and among cultivars. For sweet cherries, TA ranges from 0.7 to 1.2 g malic acid per 100 g FW (Table 17.1), with cultivars such as ‘Lapins’ showing low TA values, while ‘Sweetheart’ contains higher values. In the case of sour cherry, several studies reported a TA range of 1.4–2.9 g malic acid per 100 g FW (Damar and Ekşi, 2012). Grafe and Schuster (2014) found TA from 1.3 to 3.1 g malic acid per 100 g FW (‘Spinell’ and ‘Topas’, respectively). A similar range was determined in Portuguese (Rodrigues *et al.*, 2008), Hungarian (Papp *et al.*, 2010) and Serbian (Rakonjac *et al.*, 2010) germplasm collections.

### 17.2.3 Maturation index

The maturity index (TSS/TA ratio) is one of the major analytical measures of fruit quality, and it is widely accepted that it directly affects the perception of sweetness and flavour, and thus consumer acceptance of the cherry fruit (Crisosto *et al.*, 2003). In this sense, Guyer *et al.* (1993) observed that, as the TSS/TA ratio of cherry fruits increases, so does the consumer perception of sweetness. Compared with sweet cherry, sour cherry is characterized by a higher acidity level, resulting in lower TSS/TA ratios. For sweet cherry, reported values range between 19.0 in Turkish cultivars (Hayaloglu and Demir, 2015) to 29.0 in some Canadian sweet cherries (Girard and Kopp, 1998), while values around 40.0 have been monitored in certain cultivars (Usenik *et al.*, 2010; Serradilla *et al.*, 2012). For sour cherry, evaluation of the physicochemical composition of 33 sour cherries revealed a range in TSS/TA ratio from 5.8 to 15.3 (Wojdyto *et al.*, 2014), while in Hungarian cultivars it varied from 9.6 to 15.8 (Table 17.1). Cultivars with higher TSS/TA ratios ( $\geq 11.0$ ), contributing

to a balanced flavour, have been considered an optimal choice for fresh consumption (Papp *et al.*, 2010). The new German cultivars ‘Achat’ (12.2) and ‘Spinell’ (14.8) (Schuster *et al.*, 2014) and the new Serbian cultivar ‘Lenka’ (17.7) (Fotirić Akšić *et al.*, 2015), resulting from different breeding programmes in Germany and Serbia, were particularly selected for fresh consumption due to their sweet sour cherry taste.

### 17.2.4 Volatile compounds

In terms of sensory quality, aroma and flavour are becoming key factors that determine the choice to purchase a fruit, although the compounds that contribute to the flavour of fresh fruit comprise only 0.001–0.01% of the fruit’s fresh weight (Zhang *et al.*, 2007; Valero and Serrano, 2010). It is well known that the aroma of the fruit is the result of a complex mixture of esters, alcohols, aldehydes, ketones and terpenoid compounds (Li *et al.*, 2008; Valero and Serrano, 2010). In the case of cherries, their aroma has been studied extensively and comprises free and glycosidically volatile compounds (Girard and Kopp, 1998; Serradilla *et al.*, 2012; Wen *et al.*, 2014). Among the free volatile compounds, more than 100 have been identified such as hexanal, (E)-2-hexenal and benzaldehyde, which are among the predominant volatile flavour constituents in both sweet and sour cherries (Schmid and Grosch, 1986; Poll *et al.*, 2003; Serradilla *et al.*, 2016). In this sense, it has been reported that volatile compounds such as decanal, nonanal and (Z)-3-hexenal were identified as important odorants in ‘Lapins’, ‘Rainier’ and ‘Stella’ (Girard and Kopp, 1998). On the other hand, the aromatic carbonyl benzaldehyde has been determined at the highest level in sour cherry (Levaj *et al.*, 2010). Alcohols were the second largest class, including compounds such as benzyl alcohol, 1-hexanol and (E)-2-hexen-1-ol for sweet cherries. In contrast, according to Levaj *et al.* (2010), the alcohols identified in sour cherries, aside from 1-hexanol, were 1-butanol and 2-phenylethanol. Other compounds such as acids

have also been identified, mainly linear and branched acids, esters, monoterpenes (C10), sesquiterpenes (C15) and diterpenes (C20), in both sweet and sour cherries (Levaj *et al.*, 2010; Serradilla *et al.*, 2016). Aside from free volatile compounds, Wen *et al.* (2014) also reported that glycosidically bound aromatic compounds, integrated mainly by alcohols and terpenes, contribute markedly to the aroma of cherries.

## 17.3 Nutritional Composition

### 17.3.1 Water

Water is considered the predominant component of cherries, followed by carbohydrates, proteins and lipids (Serradilla *et al.*, 2016). The water content of sweet and sour cherry genotypes, as fleshy fruits, is around 80–83% (Serradilla *et al.*, 2016) and 81–88% (Filimon *et al.*, 2011), respectively. In general, the water content of sweet cherries is lower than that obtained from other stone fruits such as peaches with 88%, plums with 87% or apricots with 86% (USDA ARS, 2016).

### 17.3.2 Carbohydrates, proteins and lipids

Carbohydrates are the most abundant macronutrients found in cherries (Pacífico *et al.*, 2014; Bastos *et al.*, 2015). Although differences can be observed among cultivars, in general terms fruits exhibit moderate amounts of carbohydrates between 12.2 and 17.0 g per 100 g edible portion for sweet cherry, while sour cherry fruit has an average value of 12.2 g per 100 g edible portion (USDA ARS, 2016). In addition, within the genus *Prunus*, cherry fruit is a moderate source of dietary fibre, accounting for 1.3–2.1 g per 100 g edible portion (McCune *et al.*, 2011).

For sweet cherries, the protein content is between 0.8 and 1.4 g per 100 g edible portion (Serradilla *et al.*, 2016). However, for sour cherries, the protein content is below 1.0 g per 100 g edible portion (Ferretti *et al.*, 2010).

In general, the fat content of sweet and sour cherries is low and below 1.0 g per 100 g edible portion, particularly saturated fat as cherries are a cholesterol-free fruit (Ferretti *et al.*, 2010; McCune *et al.*, 2011; Pacifico *et al.*, 2014).

### 17.3.3 Sugars

Among these compounds, simple sugars (glucose, fructose and sorbitol) are the most relevant (Usenik *et al.*, 2008, 2010; Serradilla *et al.*, 2011; Ballistreri *et al.*, 2013; Pacifico *et al.*, 2014), although trace amounts of sucrose were also identified in sweet cherries, ranging from 0.1 to 1.2 mg per 100 g FW (Esti *et al.*, 2002; Usenik *et al.*, 2008; Ballistreri *et al.*, 2013). The major sugar in sweet and sour cherries is glucose, whose range varies from 6.0 to 10.0 g per 100 g FW, depending on the genotype and environmental conditions (Papp *et al.*, 2010; Ballistreri *et al.*, 2013). The second most abundant sugar is fructose. Its content ranges from 5.0 to 7.6 g per 100 g FW for sweet cherry and from 3.5 to 4.9 g per 100 g FW for sour cherry (Papp *et al.*, 2010; Ballistreri *et al.*, 2013). In fact, in both cases, these authors reported that the genotypes with a higher glucose content had also a higher fructose level. Aside from glucose and fructose, the content of sorbitol for sweet cherries ranged between 0.9 and 26.7 mg per 100 g FW, showing quantities similar to other fruits such as apples, pears, peaches and prunes (Usenik *et al.*, 2008; Ballistreri *et al.*, 2013).

### 17.3.4 Organic acids

The type of organic acid is an important factor in determining fruit acidity (Valero and Serrano, 2010), with malic acid being the principal organic acid in cherries, with values of 360.0–1277.0 mg per 100 g FW, accounting for more than 98% of the total organic acid content. It is also possible to find, as minor constituents, citric, succinic, shikimic, fumaric and oxalic acids (Usenik

*et al.*, 2008; Ballistreri *et al.*, 2013; Serradilla *et al.*, 2016). Additionally, Ballistreri *et al.* (2013) found a high correlation between the total content of organic acids and TA levels in sweet cherry, reflecting the influence of the different content of organic acids on TA.

### 17.3.5 Minerals

Sweet cherry is considered to be a good source of dietary potassium with approximately 260.0 mg potassium per 100 g edible portion (McCune *et al.*, 2011). For sour cherry, potassium is also the main mineral with 200.0 mg per 100 g edible portion. Cherries also contain other minerals in low concentrations such as calcium, phosphorus, magnesium and sodium (USDA ARS, 2016). In sweet cherries, calcium concentration ranged between 13.0 and 20.0 mg per 100 g edible portion, while phosphorus levels varied between 15.0 and 18.0 mg per 100 g edible portion, magnesium between 8.0 and 13.0 mg per 100 g edible portion, and sodium between 1.0 and 8.0 mg per 100 g edible portion. Sour cherries showed a content of calcium that ranged between 9.0 and 14.0 mg per 100 g edible portion, magnesium between 7.0 and 10.0 mg per 100 g edible portion, and phosphorus between 9.0 and 20.0 mg per 100 g edible portion (Mitić *et al.*, 2012; USDA ARS, 2016).

### 17.3.6 Vitamins

Cherries are an excellent source of vitamins, especially vitamin C (7.0–50.0 mg per 100 g edible portion), followed by vitamin E (0.1 mg per 100 g edible portion) and vitamin K (2.0 µg per 100 g edible portion) (McCune *et al.*, 2011). In addition, sour cherries are characterized by a higher content of vitamin A (64.0 mg of retinol activity equivalent (RAE) per 100 g edible portion), whereas the vitamin A content in sweet cherries is around 3.0 mg RAE per 100 g edible portion (Serradilla *et al.*, 2016).

## 17.4 Phytochemical Composition and Antioxidant Activity

### 17.4.1 Carotenoids

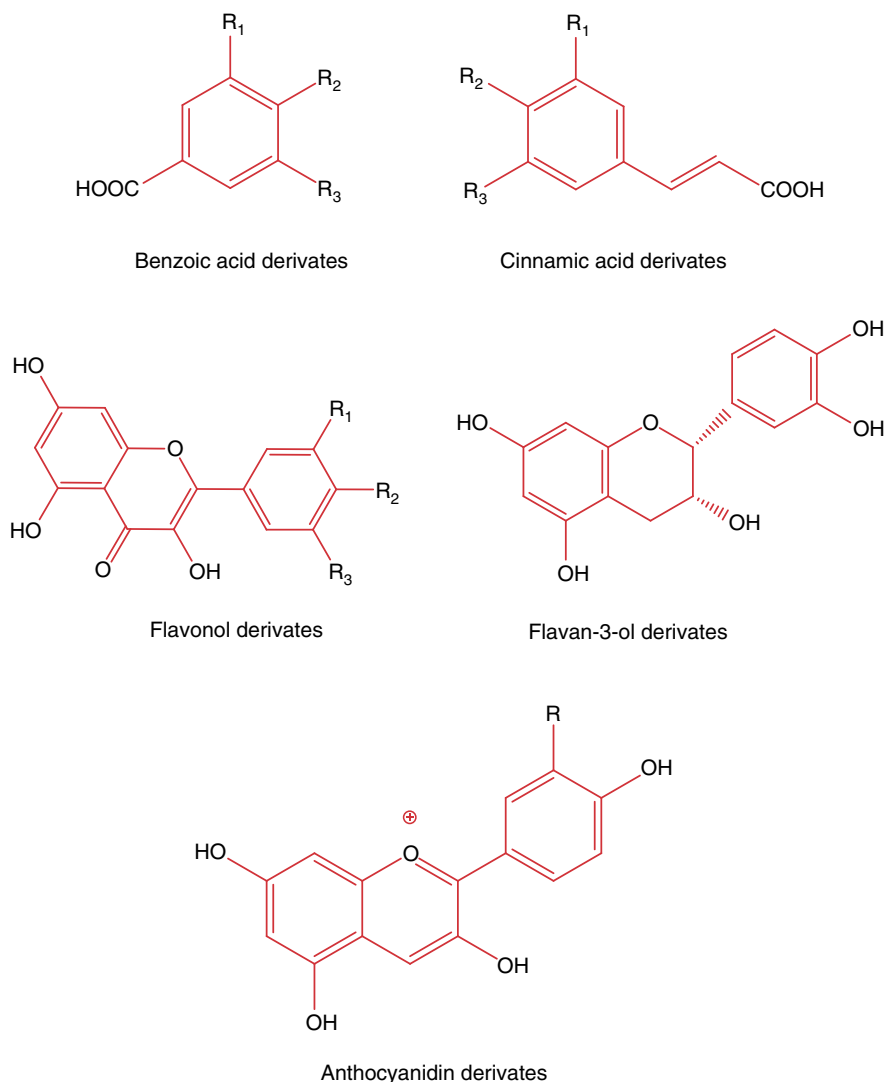
Carotenoids are the most widely distributed group of pigments naturally accumulating in large quantities, and are known for their structural diversity and various functions, including the brilliant red, orange and yellow colours of edible fruits (Valero and Serrano, 2010). Within the major phytochemicals found in sweet cherries are carotenoids (β-carotene, lutein, α-carotene, β-cryptoxanthin, zeaxanthin and phytoene). Sweet cherries contain important amounts of carotenoids, mainly β-carotene (38.0 µg per 100 g FW) and lutein/zeaxanthin (85.0 µg per 100 g FW) (Tomás-Barberán *et al.*, 2013). Sour cherries contain some carotenoids, in particular β-carotene (770.0 µg per 100 g FW), and to a lower extent lutein and zeaxanthin (85.0 µg per 100 g FW) (Ferretti *et al.*, 2010).

### 17.4.2 Phenolic compounds

Phenolic compounds, as well as their health-promoting properties, also play a key role in cherry quality attributes since they contribute to colour, taste, aroma and flavour (Tomás-Barberán and Espín, 2001). Despite its significant economic impact as a temperate fruit crop, few comprehensive studies have dealt with physicochemical and phytochemical aspects of different cultivars (Ballistreri *et al.*, 2013). Most of these studies have focused on analyses solely at harvest time, although alterations in the phytochemical content during the postharvest period have also been reported (Valero *et al.*, 2011).

Cherry polyphenols include phenolic acids (hydroxycinnamic and hydroxybenzoic acids) and flavonoids (anthocyanins, flavonols and flavan-3-ols) (Fig. 17.1). Those secondary metabolites are involved in antioxidative defence of plants against biotic and abiotic stresses such as high and low temperatures, drought, alkalinity, salinity,





**Fig. 17.1.** The main phytochemical compounds present in sweet and sour cherries. (Drawn using ChemDraw Ultra v.12.0, CambridgeSoft®.)

UV stress and pathogen attack (Viljevac *et al.*, 2012). The highest levels of total polyphenolic compounds are found in the skin of cherry fruits, followed by the flesh and pit (Chaovanalikit and Wrolstad, 2004). Current epidemiological studies strongly support a contribution of polyphenols in the prevention of cardiovascular diseases, cancers, diabetes, insomnia, obesity and osteoporosis, as well as neurodegenerative

diseases (Kang *et al.*, 2003; Kim *et al.*, 2005; Pigeon *et al.*, 2010).

A wide range of concentrations of total phenolic content (TPC) has been reported both in sweet and sour cherries (Ballistreri *et al.*, 2013; Tomás-Barberán *et al.*, 2013; Serradilla *et al.*, 2016). The most important results for both species are represented in Table 17.2, which show that sour cherry exhibits higher TPC than sweet cherry.

**Table 17.2.** Total phenolic content of sweet and sour cherries. Data are expressed on a fresh weight (FW) or dry weight basis (DW).

Species	Total phenolic content		References
	(mg per 100 g FW)	(mg per 100 g DW)	
Sweet cherry ( <i>Prunus avium</i> L.)	44.3–192.0	440.0–1309.0	Usenik <i>et al.</i> (2008); Serra <i>et al.</i> (2011); Ballistreri <i>et al.</i> (2013); Tomás-Barberán <i>et al.</i> (2013)
Sour cherry ( <i>Prunus cerasus</i> L.)	74.0–754.0	1539.0–2983.0	Kim <i>et al.</i> (2005); Bonerz <i>et al.</i> (2007); Dragović-Uzelac <i>et al.</i> (2007); Kirakosyan <i>et al.</i> (2009); Khoo <i>et al.</i> (2011); Wojdyło <i>et al.</i> (2014); Alrgei <i>et al.</i> (2016)

### Phenolic acids

Phenolic acids or phenolcarboxylic acids are types of aromatic secondary plant metabolites, widely spread throughout the plant kingdom. They contribute to food quality and organoleptic properties, and they belong to two subgroups: the hydroxybenzoic and the hydroxycinnamic acids.

Small amounts of hydroxybenzoic acids have been found in sweet cherries (Mattila *et al.*, 2006). With respect to sour cherry, Díaz-García *et al.* (2013) found gallic acid, 3,4-dihydroxybenzoic acid and vanillic acid, in accordance with the results of Chaovana-likit and Wrolstad (2004).

In contrast to hydroxybenzoic acid content, sweet cherries are rich in derivatives of hydroxycinnamic acids, which are the dominant polyphenols in sweet cherry fruit (Tomás-Barberán *et al.*, 2013; Martínez-Esplá *et al.*, 2014). The major hydroxycinnamic acids in sweet cherry are neochlorogenic and *p*-coumaroylquinic acid, followed by chlorogenic acid (Serradilla *et al.*, 2016). According to Mozetič *et al.* (2006), the ratio of neochlorogenic acid to *p*-coumaroylquinic acid is characteristic of each sweet cherry cultivar. Additionally, regarding neochlorogenic acid content, sweet cherry cultivars can be classified into three groups, the first group ranging between 40.0 and 128.0 mg per 100 g (e.g. 'Bing'), the second group between 20.0 and 40.0 mg per 100 g FW (e.g. '0900 Ziraat') and the third group ranging from 4.0 to 20.0 mg per 100 g FW (e.g. 'Sweetheart') (Ballistreri *et al.*, 2013). On the other hand, *p*-coumaroylquinic acid content

ranges from 0.8 to 131.0 mg per 100 g FW (Table 17.3) (Serradilla *et al.*, 2016). Currently, this acid is increasingly receiving attention for its health-promoting potential due to its ability to inhibit low-density lipoprotein (Tomás-Barberán *et al.*, 2013). Finally, Ballistreri *et al.* (2013) reported that chlorogenic acid concentrations in 24 sweet cherry cultivars were between 0.2 and 8.7 mg per 100 g FW.

Regarding sour cherry cultivars, the hydroxycinnamic acids found in cultivars 'Érdi Bótermő' and 'Aode' grown in Chinese agroecological conditions were neochlorogenic acid, 4-coumaroylquinic acid, caffeoylquinic acid, chlorogenic acid and 3',5'-dicafeoylquinic acid (Cao *et al.*, 2015), where neochlorogenic and chlorogenic acid were dominant. An earlier study (Kim *et al.*, 2005) showed that the amount of chlorogenic acid in sour cherries was between 0.6 and 5.8 mg per 100 g FW, while neochlorogenic acid ranged from 6.7 to 27.8 mg per 100 g FW.

Similarly, Wojdyło *et al.* (2014) determined that in almost all 33 sour cherry cultivars studied, neochlorogenic acid (~47%) was the major hydroxycinnamic acid derivative, followed by chlorogenic acid (~30%) and *p*-coumaroylquinic acid (~19%). In 'Oblačinska' sour cherry clones, chlorogenic acid, the most widespread natural plant dietary antioxidant, varied from 0.8 to 3.7 mg per 100 g FW (Alrgei *et al.*, 2016). Levaj *et al.* (2010) determined the derivatives of caffeic, *p*-coumaric and chlorogenic acid in both 'Maraska' and 'Oblačinska' sour cherry cultivars (Fig. 17.2).

**Table 17.3.** Standard phytochemical attributes of sweet and sour cherries.

Species	Anthocyanins		References	Hydroxycinnamic acids		References	Flavonols	Flavan-3-ols	References
	CY 3-O-GLU	CY 3-O-RUT		NCHL	PCQ		QUER	EPIC	
Sweet cherry ( <i>Prunus avium</i> L)	0.1–35.0 <sup>a</sup>	2.0–243.0 <sup>a</sup>	Gao and Mazza (1995); Usenik <i>et al.</i> (2008); Ballistreri <i>et al.</i> (2013)	4.0–128.0 <sup>a</sup>	0.8–131.0 <sup>a</sup>	Ballistreri <i>et al.</i> (2013); Serradilla <i>et al.</i> (2016)	2.0–6.0 <sup>a</sup>	0.4–14.8 <sup>a</sup>	Usenik <i>et al.</i> (2008); González-Gómez <i>et al.</i> (2010)
Sour cherry ( <i>Prunus cerasus</i> L)	0.9–1.3 <sup>a</sup>	9.5–17.1 <sup>a</sup>	Jakobek <i>et al.</i> (2007)	9.4–12.6 <sup>a</sup>		Mitić <i>et al.</i> (2012)	0.03–0.8 <sup>a</sup>	18.0–283.0 <sup>b</sup>	Wojdyło <i>et al.</i> (2014)
	2.0–9.9 <sup>c</sup>	35.4–85.5 <sup>c</sup>	Mitić <i>et al.</i> (2012)	212.0–998.0 <sup>c</sup>	191.0–999.0 <sup>c</sup>	Bonerz <i>et al.</i> (2007)			
	10.1 <sup>c</sup>	93.0 <sup>c</sup>	Damar and Ekşi (2012)						

CY 3-O-GLU, cyanidin 3-O-glucoside; CY 3-O-RUT, cyanidin 3-O-rutinoside; NCHL, neochlorogenic acid; PCQ, *p*-coumaroylquinic acid; QUER, quercetin; EPIC, epicatechin.

<sup>a</sup>mg per 100 g FW.

<sup>b</sup>mg per 100 g DW.

<sup>c</sup>mg L<sup>-1</sup> of juice.



**Fig. 17.2.** Sour cherries (cultivar ‘Oblačinska’).

### Flavonoids

The flavanoids or bioflavonoids are a class of plant secondary metabolites. They include anthoxanthins (flavones and flavonols), flavanones, flavanonols, flavans and anthocyanidins. They play a role in protection against UV radiation, as well as being natural pigments, enzyme inhibitors, and precursors of toxic substances, flavour components and antioxidants, and they also provide resistance to pathogens (Piccolella *et al.*, 2008). Their functionality in human health has been proved in numerous studies suggesting protective effects against cardiovascular diseases, cancers and other age-related diseases (Yao *et al.*, 2004). The main flavonoids present in cherries are provided below.

**ANTHOCYANINS.** The common anthocyanidins, which are responsible for the attractive colour of cherries, are cyanidin, pelargonidin, peonidin, delphinidin, petunidin and malvidin (Valero and Serrano, 2010). For their quantitative and qualitative analysis, the main methodology used has been the technique of high-performance liquid chromatography (HPLC) coupled to a diode array detector (DAD) or a single quadrupole mass spectrometer equipped with an atmospheric pressure electrospray ionization source (API-ES-MS) (González-Gómez *et al.*, 2010; Serra *et al.*, 2011). Anthocyanins such as cyanidin 3-*O*-rutinoside, cyanidin 3-*O*-glucoside, peonidin 3-*O*-rutinoside and pelargonidin 3-*O*-rutinoside have been reported in

sweet cherries (Gonçalves *et al.*, 2004; González-Gómez *et al.*, 2010). However, Tomás-Barberán *et al.* (2013) and Serradilla *et al.* (2016) reported that cyanidin 3-*O*-rutinoside and cyanidin 3-*O*-glucoside are the predominant anthocyanins in sweet cherries.

For sweet cherries, total anthocyanin content ranges from a few milligrams per 100 g FW in light-coloured (score of 3 on the CTIFL colour chart) to about 300 mg per 100 g FW in dark cherries (score of 5) (Gao and Mazza, 1995; Wang *et al.*, 1997; Valero and Serrano, 2010). In general, light-coloured and dark-coloured red sweet cherry cultivars contain cyanidin 3-*O*-rutinoside (2.0–243.0 mg per 100 g FW) and cyanidin 3-*O*-glucoside (0.1–35.0 mg per 100 g FW) (Table 17.3), as the primary and secondary anthocyanin, respectively (Gao and Mazza, 1995; Usenik *et al.*, 2008; Ballistreri *et al.*, 2013).

The total anthocyanin content of sour cherries was reported to be between 27.8 and 80.4 mg per 100 g FW (Blando *et al.*, 2004). However, total anthocyanin content and the anthocyanin fractions differ according to the sour cherry cultivar (Wang *et al.*, 1997; Kim *et al.*, 2005; Simunic *et al.*, 2005). Several ‘Oblačinska’ clones, studied by Alrgei *et al.* (2016), showed substantial levels of total anthocyanin content (over 100.0 mg cyanidin 3-*O*-glucoside per 100 g FW). Several types of anthocyanin compounds were also determined in sour cherries by HPLC-DAD/API-ES-MS, but the most prevalent were those that are derivatives of cyanidin (cyanidin 3-*O*-glucosylrutinoside, cyanidin 3-*O*-sophoroside, cyanidin 3-*O*-rutinoside, cyanidin 3-*O*-glucoside, cyanidin 3-*O*-xylosylrutinoside and cyanidin 3-*O*-arabinosylrutinoside) (Blando *et al.*, 2004; Chaovana-likit and Wrolstad, 2004; Kim *et al.*, 2005; Bonerz *et al.*, 2007; Cao *et al.*, 2015). According to Kirakosyan *et al.* (2009), total cyanidins in ‘Montmorency’ cherries are about 93% of total anthocyanins, while in Balaton™ (syn. ‘Újfehértói Fürtös’) they are about 94%. Finally, Mulabagal *et al.* (2009) reported that the cultivars ‘Montmorency’ and ‘Batalon’ are characterized by exhibiting cyanidin 3-*O*-glucosylrutinoside and cyanidin 3-*O*-rutinoside at a ratio of 3/1.

Peonidin 3-*O*-rutinoside, peonidin- 3-*O*-glucoside and pelargonidin 3-*O*-glucoside were also found in sour cherry fruit but in much lower concentration (Kirakosyan *et al.*, 2009), while the Hungarian sour cherry cultivars included in the study of Ficzek *et al.* (2011) showed very low concentrations of delphinidin. Jakobek *et al.* (2009) quantified the content of cyanidin 3-*O*-rutinoside (56.9 mg per 100 g FW), cyanidin 3-*O*-glucosylrutinoside (940.1 mg per 100 g FW), cyanidin 3-*O*-sophoroside (18.6 mg per 100 g FW) and cyanidin 3-*O*-glucoside (7.0 mg per 100 g FW).

Recently, the sweet cherry fruit nutritional profile has been monitored using an array of instrumental techniques, including spectrophotometric assays, HPLC and nuclear magnetic resonance (NMR) (Goulas *et al.*, 2015). In particular, NMR spectroscopy allows a rapid screening of specific primary and secondary metabolites of sweet cherries; Goulas *et al.* (2015) showed that the resonance of H-4 can be used to discriminate anthocyanins in fruit extracts as it appears at 8.2–8.6 p.p.m., a non-overcrowded region of the spectrum. The resonance of H-4 is dependent on the substitution of the anthocyanin skeleton and the discrimination of anthocyanins in a complex mixture is feasible. In a subsequent step, cyanidin 3-*O*-rutinoside was used to study the effect of pH on the chemical shift of H-4. The data indicated that the chemical shift of the diagnostic peak (H-4) is strongly influenced by pH, highlighting the need for pH adjustment of the sample. Finally, a pH value of 3.0 was selected to obtain <sup>1</sup>H-NMR spectra, since a sharp peak of H-4 was recorded and it is also the nearest pH to the actual pH of sweet cherry fruit at harvest (Goulas *et al.*, 2015).

The fact that cherries contain significant levels of anthocyanins has attracted much attention. One of the best-known properties of anthocyanins in general is their strong antioxidant activity in metabolic reactions, due to their ability to scavenge oxygen free radicals and other reactive species. Likewise, Wang *et al.* (1999) reported that sour cherry anthocyanins have an anti-inflammatory effect in cases of rheumatoid arthritis. Seeram *et al.* (2001) found that anthocyanins

originating from sour cherries have an inhibitory effect on COX-1 and COX-2 enzymes, which trigger inflammation, offering some protection against colon cancer (Kang *et al.*, 2003) and against type II diabetes by increasing insulin excretion (Jayaprakasam *et al.*, 2005). Studies have shown that numerous factors such as harvest season, variety, stage of harvesting, climatic conditions and growing season can affect the composition and concentration of individual as well as total anthocyanins (Sass-Kiss *et al.*, 2005).

**FLAVONOLS.** Flavonols are very important bioactive compounds, crucial for human health (Knekt *et al.*, 2000). A total of six flavonols have been quantified in sweet cherry fruit, with quercetin being the predominant one, fluctuating from 2.0 to 6.0 mg per 100 g FW (Table 17.3) (Usenik *et al.*, 2008; Bastos *et al.*, 2015; Serradilla *et al.*, 2016). This compound has been reported to have a great ability to act as a free-radical scavenger and therefore is associated with the prevention of degenerative diseases caused by oxidative stress, such as cardiovascular disease and cancer (Tomás-Barberán *et al.*, 2013).

In sour cherry, Kirakosyan *et al.* (2009) claimed quercetin, kaempferol and isorhamnetin rutinoside to be the main flavonol compounds. Levaj *et al.* (2010) showed that both quercetin and kaempferol were present in ‘Maraska’ (5.4 and 3.0 mg per 100 g FW, respectively) and ‘Oblačinska’ sour cherry (3.8 and 1.3 mg per 100 g FW, respectively). The same flavonols in sour cherry were determined by Jakobek *et al.* (2007), Piccolella *et al.* (2008), Ferretti *et al.* (2010) and Liu *et al.* (2011). In addition, Alrgei *et al.* (2016) determined the quantities of myricetin, pinobanksin and galangin in specific ‘Oblačinska’ sour cherry clones.

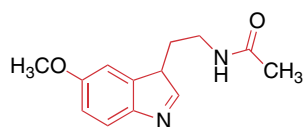
**FLAVAN-3-OLS.** In cherries, (+)-catechin and (–)-epicatechin are the main flavan-3-ols identified (Serra *et al.*, 2011). Cherry fruit stores flavan-3-ols in much lower amounts than the rest of the polyphenols. In general, for sweet cherry, (–)-epicatechin contents are higher than (+)-catechin, ranging from 0.4 mg per 100 g FW (‘Lapins’) to 15.0 mg

per 100 g FW ('Larian') (Usenik *et al.*, 2008; González-Gómez *et al.*, 2010). With respect to (+)-catechin, concentrations range from 2.9 mg per 100 g FW ('0900 Ziraat') to 9.0 mg per 100 g FW ('Noir de Guben') (Keldebek and Selli, 2011). The levels of these two compounds have been reported to be greatly influenced by agronomic and environmental conditions, as well as by genotype (Serradilla *et al.*, 2016).

For sour cherry, Usenik *et al.* (2010) reported the existence of procyanidin B2 and procyanidin dimer. As stated by Wojdyło *et al.* (2014) in a study with 33 sour cherry cultivars, procyanidin B1, procyanidin dimer, procyanidin trimer and procyanidin tetramer were found, and together ranged from 403.6 to 1215.7 mg per 100 g dry weight (DW). Besides procyanidins, Levaj *et al.* (2010) also found (+)-catechin, (–)-epicatechin and (+)-gallocatechin as monomers in both 'Maraska' and 'Oblačinska' sour cherry, which is in agreement with similar identification by Tsanova-Savova *et al.* (2005) and Chaovanalikit and Wrolstad (2004). As reported by Wojdyło *et al.* (2014), concentrations of (–)-epicatechin ranged from 18.0 to 283.0 mg per 100 g DW and of (+)-catechin from 4.0 to 116.0 mg per 100 g DW in 33 sour cherry cultivars. The highest monomer levels were found in 'Dradem', 'Meteor Korai' and 'Winer' fruits, while the lowest were found in 'Wanda', 'Lucyna' and 'Wifor' fruits. In contrast, catechin (1.4–1.6 mg per 100 g FW) is the only flavan-3-ol found in 'Érdi Böttermő' and 'Aode' (Cao *et al.*, 2015) grown in China. In one study, no flavan-3-ols were reported in sour cherry cultivars (Kim *et al.*, 2005).

### 17.4.3 Indolamines

The indolamine melatonin (MLT; *N*-acetyl-5-methoxytryptamine) is an endogenous hormone found to be present in all vertebrates (Reiter, 1993). MLT is synthesized from tryptophan via 5-hydroxytryptophan, serotonin and *N*-acetylserotonin in the vertebrate pineal gland (Fig. 17.3). MLT has been shown to possess a great number of health benefits (Reiter *et al.*, 1997). The



**Fig. 17.3.** The chemical structure of melatonin (*N*-acetyl-5-methoxytryptamine).

most well-known function of MLT in mammals is regulation of the sleep–wake cycle (Baker and Driver, 2007). Its other functions in humans range from sexual maturation to depression and antioxidative defence (Macchi and Bruce, 2004). As well as these properties, MLT has been reported to be a potent free-radical scavenger and a broad-spectrum antioxidant (Hardeland *et al.*, 2006). In addition, MLT detoxifies a variety of free radicals and reactive oxygen intermediates, including the hydroxyl radical, peroxyxynitrite anion, singlet oxygen and nitric oxide.

The presence of MLT is not restricted to the animal kingdom. This indolamine is also found in a wide variety of plants and fruits (Feng *et al.*, 2014). The MLT biosynthetic route starts with the primary metabolite shikimate; this metabolite serves as the precursor of tryptophan, which through different metabolic pathways concludes in the synthesis of melatonin (Kurkin, 2003). MLT consumed in plant products is absorbed, enters the circulation and has physiological effects via receptor- or non-receptor-mediated processes. A number of reports are available describing the positive health effects of MLT intake from cherry derivatives (Garrido *et al.*, 2009, 2012, 2013; Zhao *et al.*, 2013).

In recent years, there has been particular interest in determining and quantifying the presence of MLT in different cherry species and cultivars, since the abundance of MLT in cherries is strongly correlated with the species and fruit cultivar, and some research also indicates that MLT abundance is related to fruit maturity (Burkhardt *et al.*, 2001; González-Gómez *et al.*, 2009; Kirakosyan *et al.*, 2009). The data shown in Table 17.4 highlight the significantly higher amounts of MLT found in sour cherries.

**Table 17.4.** Melatonin concentrations reported for different sweet and sour cherry cultivars. Results are expressed as ng g<sup>-1</sup> dry weight (DW).

Species	Cultivar	Amount reported (ng g <sup>-1</sup> DW)	Reference
Sweet cherry ( <i>Prunus avium</i> L.)	'Burlat'	0.2	González-Gómez <i>et al.</i> (2009)
	'Sweetheart'	0.1	González-Gómez <i>et al.</i> (2009)
	'Pico Negro'	0.12	González-Gómez <i>et al.</i> (2009)
	'Navalinda'	0.03	González-Gómez <i>et al.</i> (2009)
	'Van'	0.01	González-Gómez <i>et al.</i> (2009)
	'Ambrunés'	0.1	González-Gómez <i>et al.</i> (2009)
	'Pico Colorado'	0.1	González-Gómez <i>et al.</i> (2009)
	Sour cherry ( <i>Prunus cerasus</i> L.)	'Montmorency'	5.6–19.6
'Montmorency'		12.3	Kirakosyan <i>et al.</i> (2009)
Balaton <sup>TMa</sup>		1.1–2.2	Burkhardt <i>et al.</i> (2001)
Balaton <sup>TMa</sup>		2.9	Kirakosyan <i>et al.</i> (2009)

<sup>a</sup>Syn. 'Újfehértói Fürtös'.

#### 17.4.4 Antioxidant activity

Antioxidant activity has been widely investigated using different methodological approaches. In cherry, antioxidant potential has been associated with ascorbic acid, phenols and anthocyanins (Chaovanalikit and Wrolstad, 2004; Serrano *et al.*, 2005, 2009). In addition, cherries are characterized by the total antioxidant activity (TAA) in both hydrophilic and lipophilic fractions by measuring the scavenging capacity of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radicals (ABTS<sup>•+</sup>) (Tomás-Barberán *et al.*, 2013). For sweet cherries, it has been reported that dark-coloured cultivars, such as 'Sonata', exhibited higher concentrations in both fractions compared with light-coloured cultivars, such as 'Brooks'. In addition, for all cultivars, hydrophilic TAA is higher than lipophilic TAA, showing that polyphenols or hydrophilic compounds are the major contributors to antioxidant activity (Tomás-Barberán *et al.*, 2013). According to Ballistreri *et al.* (2013), the TAA of 24 sweet cherry cultivars ranged from 646.0 to 3166.0 µmol Trolox equivalents (TE) per 100 g FW.

The total antioxidant capacity of 34 sour cherries was determined as between 900.0 and 6300.0 µmol TE per 100 g FW using an ABTS assay, where 'Fanal' and 'Heimanns' exhibited strong antioxidant capacity (Khoo *et al.*, 2011). As determined by Wojdyło

*et al.* (2014), the antioxidant activity of 33 sour cherry cultivars, evaluated using an oxygen radical absorbance capacity (ORAC) assay, was 8130.0–38,110.0 µmol TE 100 g DW. Among Hungarian sour cherry cultivars, 'Pipacs 1' presented an outstanding antioxidant capacity (21,850.0 µmol ascorbic acid l<sup>-1</sup>), given the fact that it is an amarelle-type sour cherry with yellowish fruit flesh, and hence with an appreciably low anthocyanin content (Papp *et al.*, 2010). Tsuda *et al.* (1994) demonstrated that cyanidin 3-*O*-glucoside shows very strong antioxidant activity. According to Heinonen *et al.* (1998), using liposomes as model membranes, anthocyanins (especially malvidin with strong antioxidant activity and cyanidin, delphinidin and pelargonidin, which have pro-oxidant activity) and hydroxycinnamates isolated from sweet cherries are more active compared with those from other berries (e.g. blackberries, red raspberries, blueberries or strawberries).

#### 17.5 Preharvest Factors Affecting Quality and Nutritional Compounds

Sweet cherry fruit is of prime importance worldwide with high commercial acceptability. However, sweet cherry is highly perishable after harvest; therefore, advanced fast precooling followed by cold storage is a

necessary postharvest tool to maintain fruit quality until consumption (Manganaris *et al.*, 2007). As mentioned earlier, the main factors determining the consumer's acceptability are TSS, acidity and colour (Crisosto *et al.*, 2003). Producers use a number of parameters to establish the optimum time for harvesting, the most reliable being skin colour (Romano *et al.*, 2006). Red colour development in sweet cherry is used as an indicator of quality and ripening, and is due to the accumulation and profile of anthocyanins (Díaz-Mula *et al.*, 2009). In addition, as mentioned earlier, in recent studies, an inverse association between fruit and vegetable intake and chronic diseases, such as different types of cancer and cardiovascular diseases, has been demonstrated in numerous epidemiological studies in which phytochemicals have been indicated to be responsible for this observed protective effect (Schreiner and Huyskens-Keil, 2006). Among these compounds, special interest has been focused on anthocyanins and other polyphenolics, carotenoids and vitamins C and E.

Consumer choice and preference for sweet cherries is influenced mainly by factors such as convenience, culture, price, appearance, taste and, in recent years, also their nutrient value and content of bioactive compounds. Accordingly, there are different preharvest factors that influence the content of bioactive compounds at the time of harvest, the most important being cultivar, temperature and light intensity, ripening stage at harvest and some preharvest treatments such as salicylate derivatives, oxalic acid and abscisic acid.

### 17.5.1 Influence of cultivar

Differences in phenolic contents were found among cultivars, with concentrations ranging between 98.0 and 200.0 mg per 100 g FW (Díaz-Mula *et al.*, 2008). 'Brooks' cherry has the lowest anthocyanin content (40.0 mg per 100 g), while 'Cristalina' shows the highest (225.0 mg per 100 g FW). The main phenolic compounds in sweet cherry fruits are anthocyanins, which also differed in concentration depending on cultivar.

Those cultivars with the lowest anthocyanins ('Brooks', 'Somerset', 'Prime Giant' and 'Sweetheart') are considered as light-coloured cultivars (score of 3 on the CTIFL colour chart), while those with the highest anthocyanin content ('Cristalina' and 'Sonata') are classified as dark-coloured (score of 5), showing a direct relationship between colour parameters and anthocyanin concentration (Díaz-Mula *et al.*, 2008). The most abundant phenolic acids in sweet cherry are derivatives of hydroxycinnamic acid such as caffeic acid and *p*-coumaric acid. The most common colourless phenolics in sweet cherries are neochlorogenic acid (3'-caffeilquinic acid) and *p*-coumaroylquinic acid (Mozetič *et al.*, 2002; Chaovanalikit and Wrolstad, 2004). The hydroxycinnamates are increasingly receiving attention for their potential health-promoting effects through their potent antioxidant action, their ability to inhibit low-density lipoprotein oxidation, and their chemopreventative properties (e.g. inhibitory effects on tumour promotion and the ability to block the formation of mutagenic compounds such as nitrosamines), as demonstrated by *in vitro* studies (Boots *et al.*, 2008; McCune *et al.*, 2011). The ability of phenolics to act as free-radical scavengers suggests that they could play a beneficial role in reducing reactive oxygen species (i.e. hydrogen peroxide, superoxide anion) associated with chronic diseases such as cardiovascular disease and cancer (Wilms *et al.*, 2005). Sweet cherry cultivars have a considerable influence on the antioxidant capacity in both hydrophilic and lipophilic extracts as measured by the scavenging capacity of ABTS<sup>•+</sup> radicals. According to Díaz-Mula *et al.* (2008), hydrophilic TAA is usually higher than lipophilic TAA for all studied cultivars (~80% of TTA in 'Cristalina' and ~50% in 'Prime Giant'), showing that the major contributors to antioxidant activity are hydrophilic compounds, such as polyphenols and anthocyanins. Antioxidant vitamins, such as tocopherols, and carotenoids are lipophilic compounds that might contribute to lipophilic TAA.

According to the National Cancer Institute (2004), sweet cherry contains important



amounts of carotenoids. Although carotenoids are another important bioactive constituent in fruits (Valero and Serrano, 2010), almost no evidence exists on their occurrence in sweet cherry. Valero *et al.* (2011) quantified carotenoids in two sweet cherry cultivars ('Prime Giant' and 'Cristalina'), and found different concentrations in both cultivars, with 'Prime Giant' having significantly higher total carotenoids (1.1 mg per 100 g FW) than 'Cristalina' (0.6 mg per 100 g FW). Leong and Oey (2012) reported individual contents in sweet cherry with concentration of 2.0 mg per 100 g DW for  $\beta$ -carotene,  $\beta$ -cryptoxanthin and  $\alpha$ -carotene, and 1.0 mg per 100 g DW for lycopene and lutein.

In sweet cherry, differences in vitamin C concentration at time of harvest have been reported. Thus, cultivar '4-70' had 28.2 mg per 100 g FW (Serrano *et al.*, 2005), while 'Souvenir', 'Samba' and 'Prime Giant' showed ascorbic acid values of 3.98, 2.30 and 5.95 mg per 100 g FW, respectively (Schmitz-Eiberger and Blanke, 2012). In sour cherry, according to Wojdyło *et al.* (2014), the content of ascorbic acid within 33 sour cherry cultivars differed greatly, ranging from 5.5 mg per 100 g FW ('Kelleris 14') to 22.1 mg per 100 g FW ('Morina'), although its content in most of the analysed cultivars was lower than 10.0 mg per 100 g FW.

### 17.5.2 Temperature and light intensity

Light intensity increases the levels of ascorbic acid, and different growing temperatures (day/night) also affect the TPC. High-temperature growth conditions (25/30°C) significantly enhance the anthocyanin and TPC (Wang, 2006). Recently, there has been increasing interest in growing cherries under plastic greenhouses, especially in cold areas. This cultivation system can influence canopy and soil temperature, and the quantity and quality of transmitted, reflected or absorbed light (Ferretti *et al.*, 2010). The highest levels of nutrients and bioactive components were found in the year characterized by the highest temperature and greatest solar radiation exposure (McCune *et al.*, 2011).

### 17.5.3 Ripening stage

Fruit ripening is a highly coordinated, genetically programmed process occurring at the later stages of fruit development and involving a series of physiological, biochemical and sensory changes leading to an edible ripe fruit with desirable quality parameters (Valero and Serrano, 2010).

In sweet cherry, the ripening process is characterized by colour changes, from green to red, which can be followed by the evolution of  $L^*$ ,  $a^*$  and  $b^*$  parameters, and are due to the accumulation and profile of anthocyanins. In fact, red colour development in sweet cherry is used as indicator of quality and ripening of fresh cherry (Esti *et al.*, 2002; Serrano *et al.*, 2005; Mozetič *et al.*, 2006). Harvesting is usually performed based on the attainment of acceptable fruit size, fruit firmness, colour and concentration of soluble solids. However, there is little available information about the changes in the content of health-promoting compounds during sweet cherry development and ripening on the tree. Serrano *et al.* (2005) reported changes in the concentrations and activities of antioxidants of sweet cherry at 14 different stages of ripeness, with total anthocyanins increasing exponentially from stage 8 to the maximum value at stage 14 (63.3 mg cyanidin equivalent activity per 100 g FW). TAA decreased from stage 1 to stage 8, and increased again from stage 8 to stage 14, coinciding with the TPC and the accumulation of anthocyanins. TAA reached its maximum activity at stage 14, with average ascorbic acid equivalent activity of 50.0 mg per 100 g FW. Thus, harvesting sweet cherries at stage 12 of ripening when the fruit reaches maximum size would support the development of the highest organoleptic, nutritional and functional quality attributes.

Gonçalves *et al.* (2004) investigated total phenolics in four cherry cultivars at two ripening stages and found the lowest total phenolics in cultivar 'Van' at the partially ripe stage (69.0 mg per 100 g FW), compared with the highest in cultivar 'Saco' at the fully ripened stage (264.0 mg per 100 g FW). Similarly, as maturity progressed

in Turkish sweet cherry (unknown cultivar), the total phenolics also increased (Mahmood *et al.*, 2013). In red-coloured fruits, total phenols generally increase during the ripening stage due to the maximal accumulation of anthocyanins and flavonols.

#### 17.5.4 Preharvest treatments

Signalling molecules, such as salicylic acid (SA) and methyl jasmonate, are endogenous plant growth substances that may play a key role in plant growth and development, and in responses to environmental stresses. The effects of SA or acetylsalicylic acid (ASA) treatments (at 0.5, 1.0 and 2.0 mM concentrations) during on-tree cherry growth and ripening were studied in ‘Sweetheart’ and ‘Sweet Late’ cultivars, and showed that treated cherries had higher concentrations of total phenolics and total anthocyanins, as well as higher antioxidant activity, in both the hydrophilic and lipophilic fractions (Giménez *et al.*, 2014). On average, treated fruit had 10–15% more phenolics, 15–20% more anthocyanins and 40–60% more antioxidant activity. The authors postulated that preharvest treatments with SA or ASA could be promising tools to improve sweet cherry quality and the health-beneficial effects for consumers.

In sour cherry (‘Cigány’), trees were sprayed with 250.0 mg L<sup>-1</sup> ethephon 1 week before the anticipated commercial harvest. Fruit from ethephon-sprayed trees had significantly lower soluble solids concentration (SSC), anthocyanin content, antioxidant activity and firmness than those from non-sprayed controls. The ethephon spray did not affect TPC, although its content tended to be higher in fruit from non-treated controls. TA, pH and SSC/TA ratio were not affected by the ethephon spray (Khorshidi and Davarynejad, 2010).

Abscisic acid (ABA) is a plant growth regulator, and plays a variety of important roles throughout the life cycle of a plant. These roles include seed development and dormancy, the plant response to environmental stresses and fruit ripening. ABA concentration is very low in unripe fruit,

but increases as the fruit ripens, so it is believed that ABA plays an important role in regulating the rate of fruit ripening. ABA application 36 days after full blossom increased the total sugar content of fruit and stimulated the accumulation of anthocyanin in sweet cherry. In contrast, ABA and ethephon applications decreased the malic acid content, whereas applications 30 days after full blossom failed to reduce the malic acid levels (Kondo and Inoue, 1977). These results suggest that ABA may be closely related to the maturation of cherry fruit, and that the effects of ABA and ethephon on maturation may vary with the time of application. It was found that ABA content increased rapidly at the straw-coloured stage and reached its highest level 4 days before commercial harvest time. During the straw-coloured stage, the application of exogenous ABA induced its accumulation, anthocyanin biosynthesis and an increase in the maturity index (TSS/TA), thereby promoting fruit ripening (Luo *et al.*, 2014).

Oxalic acid (OA), as a final metabolic product in plants, has many physiological functions, the main one being related to the induction of systemic resistance against diseases caused by fungi, bacteria and viruses by increasing defence-related enzyme activities and secondary metabolites such as phenolics. Trees of ‘Sweetheart’ and ‘Sweet Late’ sweet cherry cultivars treated with OA at 0.5, 1.0 and 2.0 mM increased fruit size at harvest, manifested by higher fruit volume and weight. Other quality parameters, such as colour and firmness, were also increased by OA treatments, which were accompanied by increases in total anthocyanins, total phenolics and antioxidant activity (Martínez-Esplá *et al.*, 2014). At the time of harvest, treated cherries had 15–20% more phenolics and 25–30% more anthocyanins, while increases of 70–80% were obtained for TAA.

#### 17.6 Postharvest Factors Affecting Quality and Nutritional Compounds

The sweet cherry horticultural production chain involves a number of steps: production,

harvesting, precooling, cooling, selection, grading, packaging, transport, distribution and consumption. Extension of the postharvest life of sweet cherry depends on three factors: (i) a reduction in dehydration and weight loss; (ii) slowing down the physiological processes of maturation and senescence; and (iii) avoiding the onset and rate of microbial growth. To control these three factors, the main tools are refrigeration and controlling the relative humidity. The optimum temperature for harvest and handling of cherries is between 10 and 20°C (outside this temperature range, more pitting is observed), while the optimum storage temperature is 0°C, with a relative humidity of 90–95% (Romano *et al.*, 2006). Thus, storage at low temperatures is the main postharvest treatment to reduce sweet cherry metabolism, maintain quality and prolong the storability in those perishable fruit and vegetables considered to be non-chilling sensitive, such as sweet cherry fruit. Some evidence exists on the changes in bioactive compounds and antioxidant activity during cold storage, although no general trends have been found. Thus, loss of health-beneficial compounds (phenolics and ascorbic acid) has been found in table grapes, broccoli, pomegranate and apple (Serrano *et al.*, 2011), in which the loss of phenolics was highly dependent on cultivar. However, increases in phytochemicals were reported for sweet cherry during cold storage, although different behaviour has been reported depending on storage temperature. Gonçalves *et al.* (2004) studied the phenolic compounds hydroxycinnamates, anthocyanins, flavonols and flavan-3-ols of 'Burlat', 'Saco', 'Summit' and 'Van' sweet cherry cultivars harvested at two different ripening stages and stored under different cold conditions. Phenolic acid content generally decreased with storage at 1–2°C and increased with storage at 15 ± 5°C. Anthocyanin levels increased at both storage temperatures, while flavonol and flavan-3-ol contents remained quite constant.

The maturity stage at harvest also determines the antioxidant potential after cold storage of sweet cherries. In a study on 11 cherry cultivars harvested at three ripening

stages (S1, S2 and S3), significant increases in anthocyanin content were found during cold storage and subsequent shelf life at 20°C, the accumulation of anthocyanins during storage being attributed to normal sweet cherry ripening (Serrano *et al.*, 2009). HPLC-DAD chromatograms revealed that in all cultivars the main anthocyanins were cyanidin 3-*O*-rutinoside, followed by cyanidin 3-*O*-glucoside and pelargonidin 3-*O*-rutinoside, which increased with ripening from S1 to S3. With respect to total phenolics, an increase in total phenolic compounds as maturity advanced was observed (from S1 to S3) for all cultivars. As mentioned above, neochlorogenic acid was the predominant hydroxycinnamic acid followed by *p*-coumaroylquinic acid, and both increased significantly from S1 to S3 and during storage.

In recent years, particular attention has been paid to the use of natural safe compounds as postharvest treatments to improve the content of bioactive compounds during storage of sweet cherries. Thus, 'Cristalina' and 'Prime Giant' cherries harvested at the commercial ripening stage and treated with SA, ASA or OA at 1 mM before storage under cold temperature showed beneficial effects on maintenance of organoleptic quality by a delay of the postharvest ripening process, manifested by lower acidity, colour changes and firmness losses. This delay was also manifested by a delay in the accumulation of total phenolics, anthocyanins and antioxidant activity (Valero *et al.*, 2011).

Another postharvest treatment with beneficial effects on reducing postharvest ripening of sweet cherry has been the use of edible coatings. In this sense, 'Sweetheart' cherry coated with sodium alginate at several concentrations (1, 3 or 5%, w/v) delayed the evolution of parameters related to postharvest ripening, such as colour, softening and loss of acidity, and reduced respiration rate. In addition, the edible coatings showed a positive effect on maintaining higher concentration of total phenolics and TAA, which decreased in control fruit associated with the over-ripening and senescence processes (Díaz-Mula *et al.*, 2012). Since the

ingestion of fruit and vegetables with higher amounts of phenolics has antioxidant activity ‘*in vivo*’ by increasing the plasma antioxidants (Fernández-Panchón *et al.*, 2008), the use of alginate as an edible coating led to fruits with higher proportion of functional properties than control ones. However, no data exist on the bioavailability and bioconversion of phenolic compounds after the intake of sweet cherry fruit, and thus more research is needed on this issue.

### 17.7 Medicinal, Traditional (Folk) and Other Usage

As described earlier, sweet cherry fruit contains fibre, vitamin C, carotenoids and anthocyanins, each of which may help play a role in cancer prevention. Medicine can be prepared from the stalks of sweet cherry drupes, which are astringent, antitussive and diuretic (Baytop, 1984). The hard, reddish-brown wood (cherry wood) is valued as a hardwood for woodturning, and for making cabinets and musical instruments (Baytop, 1984). In Turkey, sarma, a famous dish traditionally prepared from grape leaves, can also be made out of sweet cherry leaves. Sweet cherry leaves are rolled around a filling usually based on ground meat. It is found in the cuisines of the

former Ottoman Empire from the Middle East to the Balkans and central Europe.

The fruit and stem of the sour cherry are also used to produce medicine and food. Sour cherry is used for osteoarthritis, muscle pain, gout, to increase urine production, and to help digestion (McCune *et al.*, 2011). Sour cherries are eaten as a food or flavouring. Sour cherry fruit contains ingredients that reduce inflammation, protect from oxidative stress in neuronal cells (Wang *et al.*, 1999) and enhance muscle recovery (Connolly *et al.*, 2006). They also contain MLT, which helps to regulate sleep patterns (Pigeon *et al.*, 2010). With regard to sour cherry anthocyanins, *in vitro* studies have demonstrated that they are able to reduce the proliferation of human colon cancer cells in culture (Kang *et al.*, 2003).

### 17.8 Conclusions

Sweet and sour cherries are popular temperate fruits due mainly to their excellent organoleptic characteristics, especially sweet cherries. In addition, they are important sources of nutrient and bioactive food components, mainly sour cherries, and are potentially beneficial to health, and for this reason should be included as an essential part of the human diet.

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# APHIDS AS CROP PESTS

2ND EDITION

Edited by Helmut F. van Emden and Richard Harrington



# 11

## Predators, Parasitoids and Pathogens<sup>1</sup>

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### Introduction

Aphids occur in most terrestrial habitats. They are commonly attacked by predators, parasitoids and pathogens; often collectively termed Aphidophaga (Völkl *et al.*, 2007). Predators kill their prey by feeding on them. In some families of aphid predators (e.g. ladybirds), both the larvae and the adults are predaceous, whereas in other families (e.g. hover flies, lacewings, midges), only the larvae are predaceous. Among insect parasitoids, all species in the braconid subfamily Aphidiinae and some genera in the family Aphelinidae develop as endoparasitoids, with generally one larva completing development in each aphid. At the end of larval development, the aphid is killed, and the parasitoid pupates within or below the hardened cuticle of its host (the ‘mummy’). Adult wasps are free-living. Some species of fungi are entomopathogenic, infecting aphids directly through the cuticle, eventually killing the host.

In general, the impact of a natural enemy, and hence its potential contribution to a reduction in pest damage, depends on several factors. Although many aphid species are widespread and common, their density

varies over space and time. To contribute to control, a natural enemy must be effective in locating its target pest. A variety of sensory cues emanating from the aphid’s host plant, or the aphid itself, are used to locate hosts or prey over a wide range of distances. Once an aphid colony has been found, the potential voracity of a predator (or the potential fecundity of a parasitoid) plays an important role in determining the degree to which colonies will be suppressed. Patterns of resource use are also influenced by population structure and the degree of specialization. Although host-specific natural enemies are often better than polyphagous ones at finding aphid colonies, and hence are considered more effective for control, polyphagous parasitoids and predators can have a significant impact on aphid populations. For aphid pathogens, host location is largely passive; however, parameters affecting persistence, transmission and dispersal influence the potential of pathogens to infect suitable hosts.

This chapter is organized in four sections, with the first two sections dealing respectively with predators and parasitoids. The focus is on foraging behaviour and on cues triggering successful search. The third

<sup>1</sup> The authors would like to dedicate this chapter in memory of their friend and colleague, Wolfgang Völkl, who was the lead author of this chapter in the First Edition and who sadly passed away in 2015.

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section, on microbial agents, focuses on parameters affecting epizootiology; that is, the potential of a pathogen to cause an epidemic or epizootic. We discuss also the relationship between host or prey specificity and resource utilization in terms of enemy effectiveness. In the fourth section, we consider intraguild interactions and mutualistic relationships and how they may limit the effectiveness of Aphidophaga as biological control agents in the field.

## Aphid predators

### Ladybird beetles (Coleoptera: Coccinellidae) (Fig. 11.1)

#### General biology

Beetles from the family Coccinellidae comprise some 6000 species, of worldwide distribution (Seago *et al.*,

2011; Nedvěd and Kovář, 2012). They are variously called ladybirds (as in this volume), ladybugs or ladybeetles, although these names are sometimes reserved exclusively for the larger and more brightly coloured coccinellids. The family includes phytophages, mycophages and entomophages (Giorgi *et al.*, 2009; Hodek and Evans, 2012). Most entomophagous ladybirds feed on Sternorrhyncha, with many feeding predominantly on aphids. Aphidophagous species are concentrated in the tribe Coccinellini, although aphid-eating taxa can also be found in other coccinellid tribes (Giorgi *et al.*, 2009).

The lifetime fecundity of aphidophagous ladybirds varies markedly with temperature and prey type, even within a species (e.g. Papanikolaou *et al.*, 2013); this leads to widely differing estimates of lifetime fecundity. For example, reports for *Adalia bipunctata* (2-spot ladybird) feeding on aphids give



**Fig. 11.1.** Coccinellidae. (a) Eggs of *Adalia bipunctata*. (Photograph courtesy of the late S.P. Hopkin and Ardea, London.) (b) Larva of *Coccinella 7-punctata*. (Photograph courtesy of A. Stewart-Jones.) (c) Pupa of *Coccinella 7-punctata*. (Photograph courtesy of the late S.P. Hopkin.) (d) Adults of *Coccinella 7-punctata*. (Photograph courtesy of the late S.P. Hopkin and Ardea, London.)

lifetime fecundities ranging between 39 and 1535 eggs/female (Nedvěď and Honěk, 2012). Across species, it would be safe to assume a range from less than 100 to 2000 or more (data in Nedvěď and Honěk, 2012). Most aphidophagous species lay eggs in clusters (Majerus, 1994; Nedvěď and Honěk, 2012). A number of benefits accrue from this habit, possibly related to the very small size of neonate ladybird larvae, making the initial capture of prey difficult. Larvae eating unhatched, late-hatching or possible 'trophic' eggs from the same clutch survive longer and are larger, making subsequent aphid capture more likely (Banks, 1956; Hodek and Evans, 2012). Groups of neonate larvae can also feed socially (Hemptin *et al.*, 2000a; Moore *et al.*, 2012). Furthermore, fewer eggs may fall victim to predation because, when grouped together, the chemical defences they possess are more apparent to natural enemies (Agarwala and Dixon, 1993).

In keeping with other life-history characteristics, aphidophagous ladybirds develop quickly compared to coccidophagous species, as aphids constitute the more ephemeral prey (Dixon *et al.*, 1997, 2011; Borges *et al.*, 2011). Developmental times for aphidophagous ladybirds are typically in the order of several weeks (e.g. data in Dixon *et al.*, 1997). Both sexes develop at a similar rate, although adult males are smaller than females (Dixon, 2000). Within-species developmental times are affected strongly by temperature and aphid prey species (Hodek and Evans, 2012; Nedvěď and Honěk, 2012). Thus, egg to adult development of *Coccinella septempunctata* (7-spot ladybird) on *Myzus persicae nicotianae* (tobacco aphid) varies between 71.9 days at 14°C and 22.1 days at 23°C (Katsarou *et al.*, 2005), while development of the same species at 25°C varies between 17.5 days on *Myzus cerasi* (cherry blackfly) and 20.8 days on *Hyalopterus pruni*, the mealy plum aphid (Özder and Sağlam, 2003). Developmental times may even be influenced by the aphid host plant. For example, *Hippodamia variegata* exhibited developmental times varying between 12.6 days and 14.5 days on *Aphis gossypii* (cotton or melon aphid) from five different host plants (Wu *et al.*, 2010). Other parameters, including mortality and adult size, are also affected by rearing temperature and diet (Hodek and Evans, 2012; Nedvěď and Honěk, 2012).

The number of generations per year is limited to one at high latitudes, but increases as latitude decreases towards the tropics. Ladybirds in temperate regions typically diapause in winter, while ladybirds in areas with hot or dry seasons may aestivate

at those times: adults are generally the dormant life-history stage (Hodek, 2012).

Ladybirds have a variety of natural enemies. Many generalist predators avoid eating ladybirds due to their chemical defences, which are comprised of inter-specifically variable alkaloids and are present in all life-history stages (Brakefield, 1985; Ceryngier *et al.*, 2012). Populations of immature ladybirds exhibit high levels of cannibalism, especially if aphids are in short supply (Mills, 1982; Schellhorn and Andow, 1999), and may also fall victim to intraguild predation and aphid-tending ants (see below). Ladybirds are also attacked by a variety of specialized parasites, including dipteran and hymenopteran parasitoids, mites, fungi and bacteria, which can reach very high prevalences (Ceryngier *et al.*, 2012). Particularly noteworthy are the group of so-called 'male-killing' bacterial endosymbionts, which are transmitted cytoplasmically through the female line. They kill male eggs before hatching, a strategy that enhances female (and their own) survival at the expense of males (Majerus and Hurst, 1997; Ceryngier *et al.*, 2012).

### **Adult and larval foraging for aphids**

How adult ladybirds find aphid-bearing plants over long distances remains poorly understood, but cues are probably visual, such as shape and colour, and olfactory, from either the aphid or the plant (e.g. Bahlai *et al.*, 2008). Experience may play a role in the effective use of these cues (Wang *et al.*, 2015). Larvae that begin their life in a suitable habitat chosen by their mother do not forage over long distances, although they may move between adjacent plants via the ground or overlapping leaves (e.g. Banks, 1957).

On the plant, a number of behaviours bring adult and larval ladybirds into contact with their aphid prey. Ladybirds, like aphids, exhibit positive phototaxis and negative geotaxis; their search is concentrated on leaf edges, stems and particularly veins, the latter being a focus for aphid colonies (Banks, 1957; Frazer and McGregor, 1994; Hodek and Evans, 2012). Aphid honeydew is an arrestant stimulus (Carter and Dixon, 1984), ensuring that ladybirds remain in areas inhabited by prey. Plant architecture influences the efficiency of ladybird foraging if it makes aphids more difficult to detect or catch (Kareiva, 1990; Clark and Messina, 1998). Plants with waxy surfaces may be difficult to walk on, while leaf hairs or trichomes impede movement (Shah, 1982).

Some studies suggest that visual or olfactory detection by adults or larvae occurs over only short

distances of a centimetre or so (Nakamuta, 1984a; Hemptinne *et al.*, 2000a). Aphid body fluid stimulates feeding in adults (Nakamuta, 1984b). After aphid encounter, adults and larvae switch to intensive searching behaviour whereby they search the immediate area more slowly and with frequent turns (Banks, 1957; Hodek and Evans, 2012). Larvae of *Ceratomegilla undecimnotata* are highly variable in their intensive search behaviour, which can occur prior to finding an aphid, be delayed, or not occur at all after aphid capture (Ferran *et al.*, 1994). Flexibility in intensive searching behaviour is likely to be an adaptation to variable conditions, particularly prey distribution (Ferran *et al.*, 1994; Dixon, 2000).

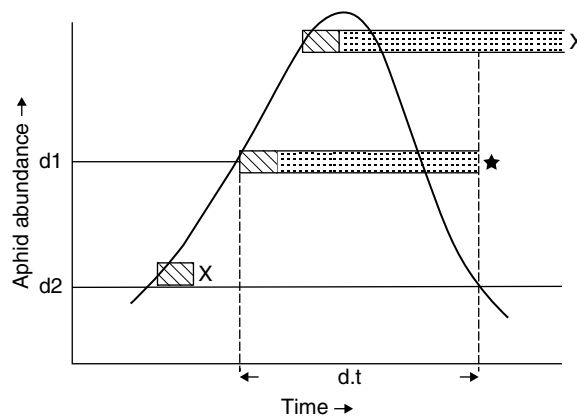
### Oviposition and predatory behaviour in aphid patches

A patch of aphids is only suitable for ladybird oviposition for a short period early in its development (the so-called ‘egg window’) (Fig. 11.2). If ladybirds oviposit too early, then there are insufficient aphids to support the developing larvae, while if ladybirds oviposit too late, the aphids will not persist for long enough at high densities to allow the larvae to reach adulthood (Hemptinne *et al.*, 1992).

It appears that the minimum density of aphids necessary for ladybird reproduction to begin is determined by the relative size of the aphid and ladybird species involved (Sloggett, 2008a). Small aphid species support oviposition by small ladybirds

at lower densities than large ladybirds, because the minimum food required to support egg production in small ladybirds is lower. However, because small ladybirds cannot catch the older life stages of large aphid species, larger ladybirds can reproduce earlier at lower total densities of large aphids. A number of studies with different sized aphid species show the same size-related pattern of temporal variability in ladybird reproduction (Sloggett, 2008a).

Although a few studies have suggested that cessation of oviposition is associated with prey-related cues (e.g. Johki *et al.*, 1988), such cues appear to be of very limited importance relative to cues from the ladybirds themselves (Hemptinne *et al.*, 2000b). When ladybird larvae are already present on a plant, subsequent clutches of eggs are at risk from intraguild predation and cannibalism (Hemptinne *et al.*, 1992; Dixon, 2000). Females avoid ovipositing when conspecific, and sometimes allospecific, ladybird larvae are present (Hemptinne and Dixon, 1991; Seagraves, 2009). This is mediated by chemical cues from larvae on the plant surface; these cues reduce the female’s tendency to oviposit and increase her activity, thereby encouraging her to leave the plant (Růžička, 1997; Doumbia *et al.*, 1998; Laubertie *et al.*, 2006). Sentis *et al.* (2015) further showed that temperature influences the emission of these chemical cues from larvae, which in turn changes the oviposition behaviour of conspecific females. Such temperature-mediated effects on chemical communication would likely influence population processes in coccinellids.



**Fig. 11.2.** The advantage of ladybird beetles synchronizing their reproductive effort with the early development of aphid populations. Dashed bars correspond to egg laying and dotted bars indicate larval development. Open means that larvae cannot complete their growth. (X) unsuccessful, (★) successful reproductive effort; d1 and d2 = threshold densities for the survival of first- and fourth-instar larvae, respectively; d.t. = developmental time. (From Hemptinne *et al.*, 1992.)

### **Prey specificity**

Aphidophagous ladybird species vary markedly in aphid prey specificity. Some ladybirds only feed on a very few aphid species: *Myzia oblongoguttata* (striped ladybird) is restricted to conifer aphids, and *Anisosticta novemdecimpunctata* (water ladybird) feeds mainly on *H. pruni* in *Phragmites* spp. reed beds. In contrast, some ladybirds are generalists, such as *A. bipunctata* and *C. septempunctata*, which feed on hundreds of different aphid species (Majerus, 1994). Complex relationships occur between diet and habitat; while dietary specialists may be restricted to one or a few plant species, some habitat specialists, such as the myrmecophile *Coccinella magnifica*, consume many different aphid species (Sloggett, 2008b).

Conventionally, aphids have been divided into ‘essential prey’, which support oviposition and development, ‘alternative prey’, which can be consumed but are unsuitable for reproduction, and ‘rejected prey’, which are unacceptable (Hodek and Evans, 2012). However, many prey species that are not consumed in the wild will serve as essential prey in the laboratory (see Majerus, 1994). Furthermore, transitional cases exist where prey species are marginally suitable for reproduction and are, therefore, difficult to categorize (Hodek and Evans, 2012).

Although aphids are the primary diet of aphidophagous ladybirds, other food items may form a significant part of their diet. These include other Sternorrhyncha, other insects including Lepidoptera, fungal spores and plant material, especially fruit and pollen (Evans, 2009; Lundgren, 2009; Hodek and Evans, 2012). Alternative food can have positive effects on oviposition, larval growth and survival, when aphids are limited (Evans *et al.*, 1999; Kalaskar and Evans, 2001). Many aphidophagous ladybirds utilize these alternative foods, as well as alternative aphid prey, during periods when essential aphid prey are scarce, such as early spring or late summer in temperate regions (Sloggett and Majerus, 2000a; Hodek and Evans, 2012).

### **Ladybirds, aphid populations and aphid control**

The impact of aphidophagous ladybirds on aphid populations remains controversial. It has been argued that ladybirds do not reproduce for long enough in a patch to provide adequate aphid control.

This is because their generation time is long relative to their prey, and because aphids are patchily distributed (Hemptinne and Dixon, 1997; Kindlmann and Dixon, 2001). Additionally, cannibalism and intraguild predation can further limit aphid control. None the less, many practitioners still consider that ladybirds play an important role in aphid population control (e.g. Obrycki *et al.*, 2009), although their ‘popularity’ has declined in recent years (Michaud, 2012). The majority of aphidophagous ladybirds considered important for biological control are generalists; but their tendency to move between prey species and habitats may limit their utility (Sloggett *et al.*, 2008) and can lead to unintended non-target effects (Evans *et al.*, 2011). In particular, the introduction of exotic ladybirds to new geographic regions for aphid control may have led to declines in native species, through competition and/or intraguild predation (Evans *et al.*, 2011).

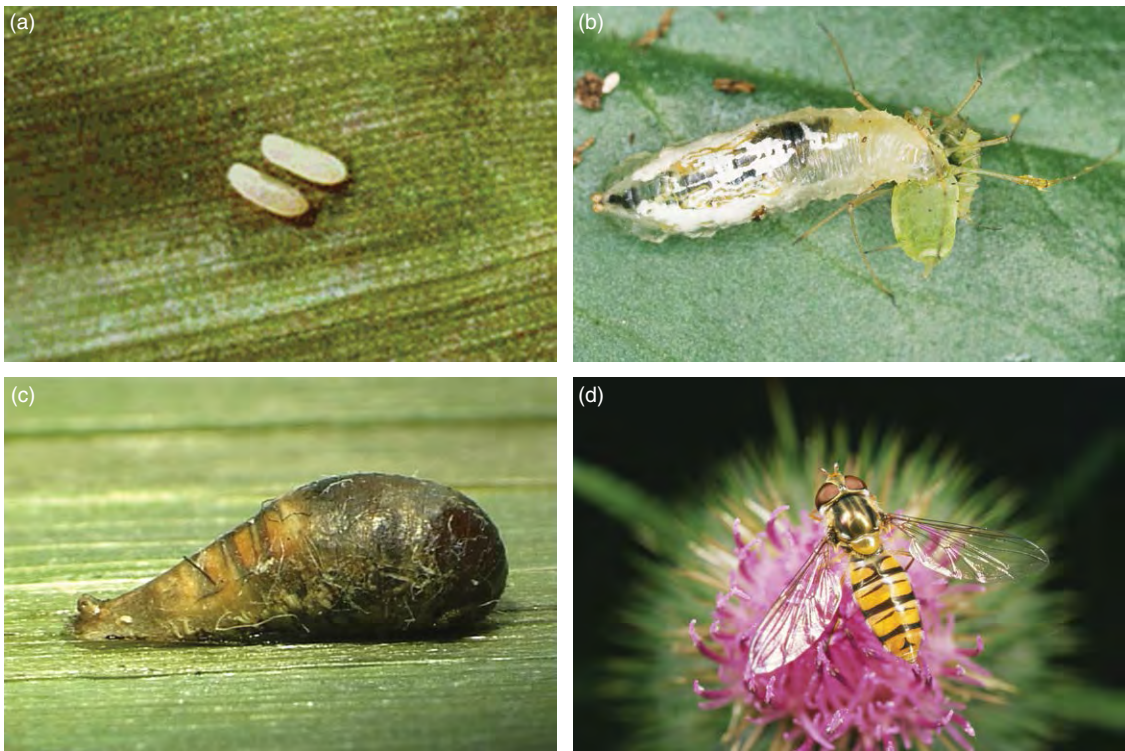
### **Syrphidae (hover flies) (Fig. 11.3)**

#### **General biology**

Hover flies are one of the largest dipteran families. Larvae of about one-third of hover fly species in the subfamily Syrphinae are predators of Sternorrhyncha, usually aphids. Adults are active diurnal flower visitors and feed on pollen and nectar (Rotheray, 1989); they mimic bees and wasps.

Adult hover flies are characteristically highly fecund. In *Episyrphus balteatus*, the average lifetime production of eggs per female ranges between 2000 and 4500 (Branquart and Hemptinne, 2000). Larvae hatch after 2–5 days and almost immediately start feeding on surrounding aphids. Larvae feed by puncturing the aphid cuticle and sucking out the contents. There are three larval stages; the final instar pupates inside a puparium, usually located on the plant where the last prey was consumed. Developmental times vary between species and are influenced by prey species and temperature.

Most species are univoltine in temperate regions, while multivoltinism is common in the tropics. In northern Europe, several species are partially migratory: some individuals remain in their summer habitats and overwinter at larval, pupal or adult stages, while the rest of the population migrates over long distances to southern Europe (Raymond *et al.*, 2013).



**Fig. 11.3.** Syrphidae. (a) Syrphid eggs, species not known. (Photograph courtesy of B. Freier and the Biologische Bundesanstalt für Land- und Forstwirtschaft, Kleinmachnow, Germany.) (b) Larva of *Episyrrhus balteatus*. (Photograph courtesy of the late S.P. Hopkin and Ardea, London.) (c) Puparium of *Episyrrhus balteatus*. (Photograph courtesy of U. Wyss.) (d) Adult *Episyrrhus balteatus*. (Photograph courtesy of the late S.P. Hopkin and Ardea, London.)

### Oviposition site selection

Oviposition in hover flies is elicited by olfactory and visual cues. Females of *Eupeodes corollae* and *E. balteatus* respond positively to stimuli from honeydew, and probably also from aphid siphunculus secretions. These stimuli may act both as long-distance kairomones and oviposition stimuli following prey location (Budenberg and Powell, 1992; Bargaen *et al.*, 1998; Sutherland *et al.*, 2001). Additionally, females of *E. corollae* respond to plant structural characteristics, having a preference for vertical over horizontal surfaces and darker over lighter strips (Chambers, 1988). Eggs are often laid singly, either close to or within aphid colonies, although some species lay eggs in batches distant from the colony, or even on uninfested plants (Chambers, 1988). In the latter case, young larvae may survive by cannibalizing conspecific eggs.

Aphid colony size has an important influence on oviposition site selection. Since larvae have a limited dispersal capability, the female's decision on oviposition site is crucial to the survival of her offspring. Hover fly females adjust their egg number in relation to aphid density, behaviour considered adaptive since it both secures larval survival and optimizes the female's search effort. Generally, the number of eggs deposited increases with aphid colony size (Bargaen *et al.*, 1998; Sutherland *et al.*, 2001). In contrast, there are reports that females of many hover fly species prefer smaller aphid colonies, or aphid colonies with a high proportion of early instars, for oviposition (e.g. Ito and Iwao, 1977; Hemptinne *et al.*, 1993). These females may be avoiding high aphid densities because large colonies are more likely to be subject to increased aphid emigration, rather than to continued colony growth, the latter being essential to ensure the survival of hover fly offspring (Kan, 1988). Hover fly females



also lay fewer eggs in aphid colonies where conspecific larvae are already present (Hempton *et al.*, 1993).

### **Prey specificity and larval foraging**

Field and laboratory observations suggest that hover fly larvae attack a broad range of prey types (Chambers, 1988; Gilbert and Owen, 1990), but there are also truly specialized species, such as *Eupeodes nielsenii*, which consumes only a few aphid species on pine (Laska, 1978). In polyphagous species, different prey in the diet may influence developmental time and pupal weight, which in turn may influence adult fecundity (Sadeghi and Gilbert, 2000).

Hover fly larvae are active between dusk and dawn. Their search is characterized by casting behaviour (Chambers, 1988) in which the hind body remains attached to the substrate while the anterior end is extended forward and laterally until a prey item is contacted. Evidence for the importance of olfactory and gustatory cues for larval foraging behaviour is inconsistent. Chambers (1988) assumed that hover fly larvae recognized aphids only by tactile cues and that they did not use directed cues for prey location. In contrast, Borgen *et al.* (1998) found that first-instar larvae of *E. balteatus* exhibited a directed search over short distances and responded to volatile olfactory cues from aphids. Rapid prey location would be especially important for first-instar larvae hatching from eggs that had been deposited at a distance from the aphid colony.

In the laboratory, hover fly larvae show great voracity. Single larvae of Syrphini consume between 250 and 500 aphids, depending on aphid size and temperature (e.g. Chambers, 1988; Soleyman-Nezhadian and Laughlin, 1998). Larvae of Melanostomini, which are considerably smaller, consume between 135 and 150 aphids (e.g. Chambers, 1988). In all studies, third-instar larvae consumed more than 80% of aphids captured. Larvae of this age class were also most successful and efficient at capturing prey. When provided with an excess of aphids, hover fly larvae often killed more than they required for development and only partially consumed killed aphids (Chambers, 1988).

### **Hover flies and aphid populations**

Hover flies can be important antagonists of aphids, particularly in cereals (Hagen *et al.*, 1999). Striking aphid population reduction was observed when high levels of hover fly oviposition and larval

hatching occurred early in the season, before aphid population growth accelerated (Tenhumberg and Poehling, 1995). Besides climatic factors, their effectiveness is limited by slow immigration rates of adults into fields and in simple landscapes (Haenke *et al.*, 2009; Chaplin-Kramer and Kremen, 2012). Since adults are flower visitors, their foraging activity in crops can be enhanced when field margins provide a continuous supply of flowers such as Asteraceae and Apiaceae, which have easily accessible pollen (Ruppert and Molthan, 1991; Hogg *et al.*, 2011). Since cereal fields are usually characterized by a shortage of food for flower visitors, alternative agricultural practices that favour wild flowers (e.g. set-aside, herbicide-free buffer zones, conservation strips (Chapter 21, this volume) may attract adult hover flies. Besides their impact on cereal aphids, hover flies also reduce aphid populations in citrus and apple orchards (Tracewski *et al.*, 1984; Michaud and Belliure, 2000), and even in tropical crops such as taro and banana (Stechmann and Völkl, 1990).

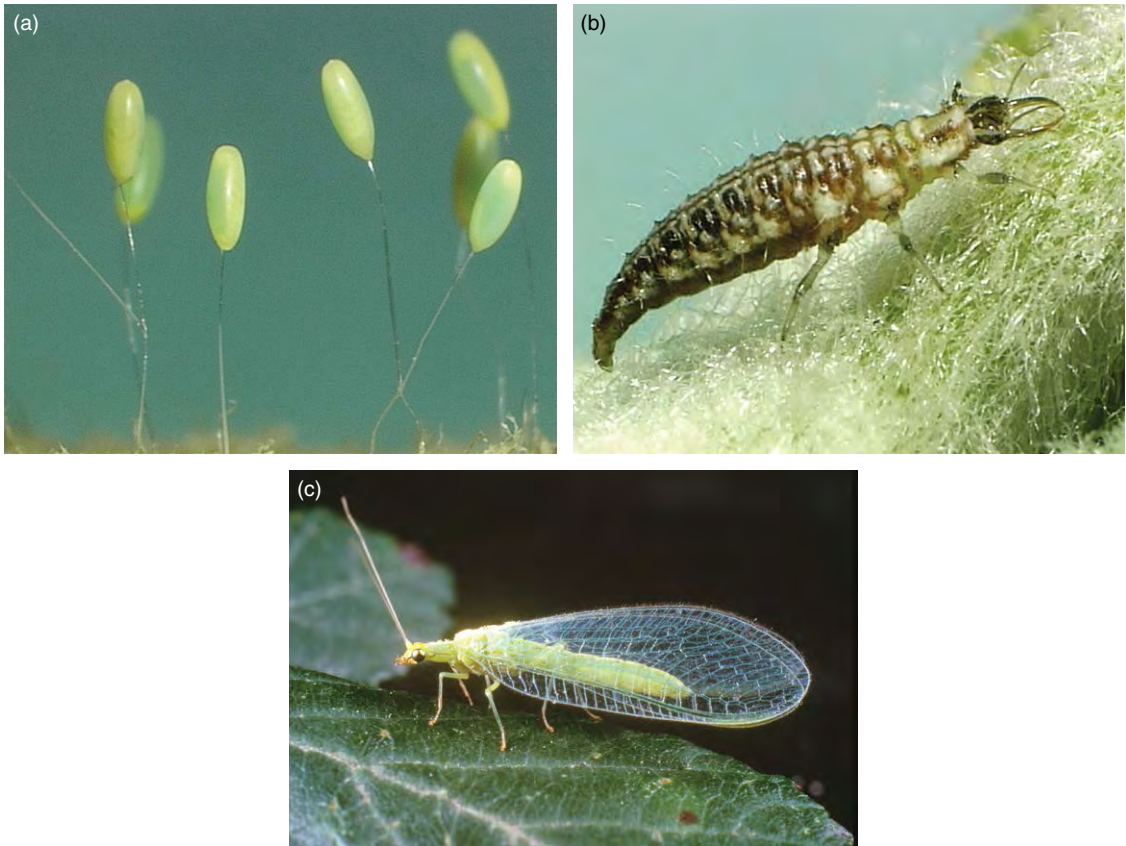
## **Chrysopidae and Hemerobiidae (lacewings)** **(Fig. 11.4)**

### **General biology**

Lacewings (Neuroptera) are polyphagous predators feeding mainly on soft-bodied insects. Species of Chrysopidae and Hemerobiidae all commonly predate aphids, and some species in the Coniopterygidae have also been recorded feeding on aphids, particularly on conifers (Canard *et al.*, 1984; New, 1988).

The eggs of many lacewing species have thin hyaline stalks (egg pedicels), although this is absent in other species. The pedicel protects eggs and newly hatched larvae from cannibalism and intraguild predation (Růžička, 1997).

Lifetime fecundity in chrysopid species varies between 150 and 600 eggs/female and seems to be greatest in hemerobiids (600–1500 eggs) (Canard *et al.*, 1984; New, 1988). Between 15°C and 25°C, developmental times range between 6 and 12 days for hemerobiids and between 15 and 30 days for chrysopids (Canard *et al.*, 1984; New, 1988; Liu and Chen, 2001; Michaud, 2001). In both families, there are three larval instars; pupation takes place within a globular cocoon, which is attached to the plant surface. In temperate regions, most lacewings are multivoltine (Tauber and Tauber, 1982), although the biology of many species has received



**Fig. 11.4.** Chrysopidae. (a) Eggs of *Chrysoperla carnea*; (b) larva of *Chrysoperla carnea*. (Both photographs courtesy of U. Wyss.) (c) Adult of *Chrysoperla carnea*. (Photograph courtesy of Rothamsted Research, Harpenden, UK.)

little research attention. Many lacewing species enter winter diapause (hibernation) either as adults (such as *Chrysoperla carnea* – common green lacewing – or *Micromus angulatus*), pupae, or even prepupae (New, 1988).

Chrysopid and hemerobiid larvae are very active predators, as are most of the adults. Some adult chrysopids, such as *C. carnea*, feed on nectar, yeasts, pollen and honeydew, the latter of which may attract them into aphid colonies. Larval voracity depends on prey size and temperature. Michaud (2001) reported a relationship between food consumption and developmental time of larval *Chrysoperla plorabunda*, which consumed between 130 and 1650 *Aphis* (*Toxoptera*) *citricidus*, depending on aphid instar. *Chrysoperla carnea* larvae also responded to changes in prey species, consuming more *A. gossypii* (292) and *M. persicae* (273) than *Lipaphis erysimi* (146) (Liu and Chen, 2001).

#### **Adult foraging and oviposition behaviour**

Chrysopid larvae are generalist predators. For example, Herard (1986) reported that *C. carnea* fed on more than 70 prey species from five insect orders including aphids, psyllids, mealybugs, leafhoppers and whiteflies. Many chrysopid species are attracted to cues associated with the presence of aphids. In laboratory experiments, *C. carnea* responded positively to volatile breakdown products of the amino acid, tryptophan, which is a common component of aphid honeydew (van Emden and Hagen, 1976), to aphid sex pheromone and to (*E*)- $\beta$ -farnesene, the alarm pheromone of aphids (Zhu *et al.*, 1999). *Chrysopa pallens* showed a positive response to aphid sex pheromone but not to (*E*)- $\beta$ -farnesene (Boo *et al.*, 1998). Plant volatiles, such as  $\beta$ -caryophyllene and 2-phenylethanol are also attractive (Zhu *et al.*, 1999). Furthermore, larvae of various *Chrysopa* species

mark the substrate with an oviposition-detering pheromone, which significantly reduces both intra- and interspecific oviposition activity (Růžička, 1996).

Having selected suitable areas on a given plant, the females of many neuropterans do not search specifically for an oviposition site in the direct vicinity of an aphid colony (Coderre *et al.*, 1987). For example, *Chrysopa oculata* (goldeneyed lacewing) only laid eggs on maize plants if there were aphids present; however, most eggs were found on leaves without aphid colonies. This suggests that newly hatched larvae must search intensively for prey (Coderre *et al.*, 1987). Similarly, random egg laying was observed in some other chrysopid species, such as *C. carnea*, whose adults feed on honeydew (Duelli, 1984).

The first bottleneck in larval foraging is the interval between eclosion from the egg and the first prey encounter. During this period, many first-instar larvae die of starvation or predation. Plant structures such as dense trichomes dislodge small larvae (Rosenheim *et al.*, 1999), thereby contributing to early larval mortality and making such plants less suitable for lacewing development.

### Larval foraging

Larval foraging behaviour can be modified strongly by foliage density and plant architecture, as these affect prey accessibility. Clark and Messina (1998) found that *C. carnea* was more effective in locating *Diuraphis noxia* (Russian wheat aphid) on Indian ricegrass (*Oryzopsis hymenoides*) (which is characterized by narrow, linear leaves) than on crested wheatgrass (*Agropyron desertorum*) with its flat, broad leaves.

This difference may explain the greater effectiveness of *C. carnea* in eliminating field-cage populations of *D. noxia* on rice grass than on wheatgrass (Messina *et al.*, 1997). Similarly, the presence of alternative prey reduced foraging by *C. plorabunda* larvae on *D. noxia*, even on easily accessible sites (Bergeson and Messina, 1998). Larvae of *C. plorabunda* were also more effective against *D. noxia* on resistant wheat plants than on susceptible ones (Messina and Sørensen, 2001), suggesting that even a modest reduction in aphid population growth might produce synergistic effects for pest reduction by natural enemies (van Emden, 1986).

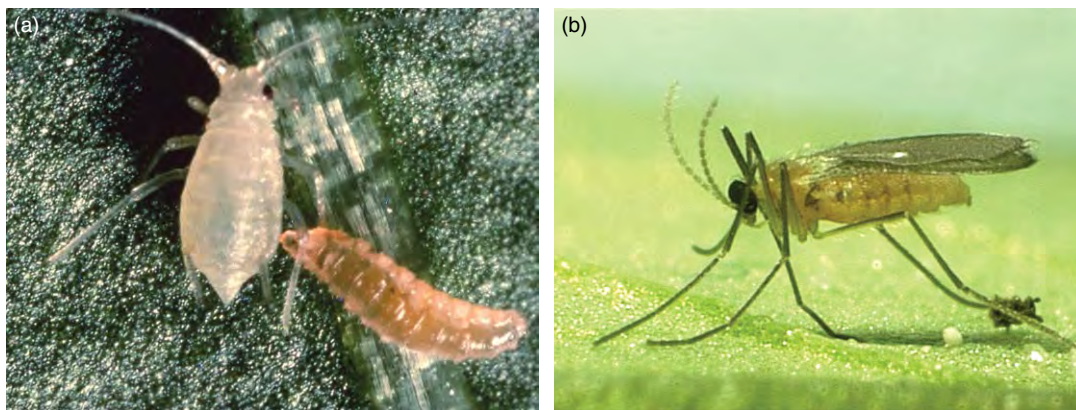
### Lacewings and aphid populations

The role of Neuroptera in reducing aphid populations depends heavily on local conditions. Chrysopids have the potential to reduce aphid numbers significantly (New, 1988), but there are only a few studies dealing with their effectiveness on aphid populations. The use of lacewings in biological control has been through augmentation and conservation, for they are open to manipulation, making them particularly successful in closed environments such as greenhouses. Research is needed to determine the conditions under which lacewings are most effective against key aphid pests.

## Cecidomyiidae (predatory midges) (Fig. 11.5)

### General biology

Within the dipteran family Cecidomyiidae, there are at least five predatory species in the genera



**Fig. 11.5.** Cecidomyiidae. (a) Larva of *Aphidoletes aphidimyza*. (Photograph courtesy of J. Bennison and ADAS.) (b) Adult of *Aphidoletes aphidimyza*. (Photograph courtesy of U. Wyss.)

*Aphidoletes* and *Monobremia* whose larvae prey exclusively on aphids, while the adults feed on nectar or honeydew (Harris, 1973; Nijveldt, 1988). *Aphidoletes aphidimyza* is commonly used in biological control programmes (van Schelt and Mulder, 2000). Adult flies are nocturnal (Nijveldt, 1988). The average number of eggs laid per female during her lifetime ranges between 50 and 150; fecundity depends both on larval and adult nutrition and on geographic origin (Havelka and Zemek, 1999). Larvae hatch after 2–4 days and almost immediately start feeding on aphids. There are three larval stages; the final instar pupates in soil. Developmental time varies with temperature and nutritional condition, ranging between approximately 30 and 55 days from egg to adult (Harris, 1973; Nijveldt, 1988; Havelka and Zemek, 1999). In central Europe, there are two or three generations per year (Harris, 1973). *Aphidoletes aphidimyza* hibernates in the pupal stage in the soil.

#### **Oviposition behaviour**

Females lay their eggs singly or in small clusters on foliage, usually within or close to aphid colonies. Females can discriminate between plant species or varieties, and especially between infested and uninfested plants (Mansour, 1975, 1976). The latter capability is crucial for *A. aphidimyza* survival, because neonate larvae cannot detect prey unless they are very close to them, and will die from starvation if they are more than 63 mm from food (Wilbert, 1973). Furthermore, ovipositing females respond to morphological host-plant characteristics such as leaf pubescence (Lucas and Brodeur, 1999). Evidence for a female response to varying aphid density is inconsistent, though positive correlations between clutch size and aphid density have been reported (Wilbert, 1973; Nijveldt, 1988; Lucas and Brodeur, 1999 – but see also Stewart and Walde, 1997). Additionally, Růžička and Havelka (1998) found that *A. aphidimyza* larvae secreted a species-specific, oviposition-detering pheromone that significantly reduced oviposition in colonies already being attacked.

#### **Prey specificity and larval feeding**

Larvae of *A. aphidimyza* feed on a variety of aphid species, mainly on herbaceous plants or deciduous trees (Harris, 1973; Nijveldt, 1988). The number of aphids killed during the larval stage varies

considerably. One larva needs a minimum of seven adult aphids to complete its life cycle, but may attack and partially consume as many as 80, depending on aphid size and environmental conditions such as humidity (e.g. Nijveldt, 1988). Thus, larvae may kill more aphids than they need for their development (Nijveldt, 1988), enhancing their success in biological control programmes, especially in glass-house systems.

### **Other arthropod predators**

#### **Predatory bugs**

**ANTHOCORIDAE (FLOWER BUGS) (FIG. 11.6).** Within the Heteropteran family Anthocoridae (Hemiptera: Heteroptera), species in the genera *Anthocoris* and *Orius* are common generalist predators. For example, the prey of *Anthocoris nemorum* (common flower bug) in Europe includes 34 insect species in four orders, and five mite species (Herard, 1986). Anthocorids, or flower bugs, are mainly diurnal. Females lay their eggs singly just beneath the epidermis of the prey's host plant. Mean total egg numbers of 50/female are common, but they may lay up to 200 (Hodgson and Aveling, 1988; Lattin, 1999). There are five nymphal stages. Their developmental time is influenced strongly by temperature, ranging from between 22 days at 20°C and 53 days at 14°C in *A. nemorum*. In *A. nemorum*, the number of generations per year depends on climate; the species is univoltine in Scotland, bivoltine in England and has at least three generations



**Fig. 11.6.** Anthocoridae. Adult *Anthocoris nemorum*. (Photograph courtesy of U. Wyss.)

in France. Anthocorid bugs usually hibernate as adults in leaf litter, under bark, or in hollow stems (Parker, 1975).

As with many other aphid predators, anthocorid females may use herbivore-induced volatiles for prey location (Dwumfour, 1992; Scutareanu *et al.*, 1997). During development, *Anthocoris* nymphs consume between 60 and 240 aphids, depending on aphid size and temperature (Hodgson and Aveling, 1988).

Anthocorid bugs are very mobile and respond rapidly to changes in prey population, thereby aggregating in areas with high prey densities (Hodgson and Aveling, 1988). In such situations, both nymphs and adults contribute to a significant reduction in aphid numbers and improved biological control (reviewed by Hagen *et al.*, 1999; see also recent work by Desneux *et al.*, 2006).

**OTHER BUGS.** Nabid and mirid bugs (Heteroptera: Nabidae and Miridae) are also aphid predators. These, like anthocorids, benefit from a diversified landscape (Müller and Godfray, 1999) and may have some impact on cereal aphid populations. Predatory bugs are considered as ‘true’ top predators that may disrupt biological control through intraguild predation (see below).

### ***Chamaemyiidae (aphid flies)***

This is a small dipteran family from the Brachycera whose larvae feed exclusively on aphids (Sunderland, 1988); *Leucopis* is perhaps the commonest genus. Adult flies are diurnal. Females lay eggs singly, close to or within an aphid colony, often within folded leaves (Gaimari and Turner, 1997). First instars usually attack aphids that walk over them. Second- and third-instar larvae search more actively for prey. Puparia are usually formed within the aphid colony. Larvae and puparia can be distinguished from equivalent hover fly developmental stages by the two distal siphons. Most chamaemyiid flies are habitat specialists (e.g. conifer forests) and/or associated with only a few aphid species (Tracewski *et al.*, 1984; Sunderland, 1988; Mizuno *et al.*, 1997). Aphid flies were recorded as particularly abundant predators in New England apple orchards (Tracewski *et al.*, 1984) and in Florida citrus orchards (Michaud and Belliure, 2000), where they might have played an important role in aphid population regulation.

## **Polyphagous predators**

Aphids are attacked by a variety of polyphagous predators such as carabid beetles (Coleoptera: Carabidae), wolf spiders (Araneae: Lycosidae) and, to a lesser extent, rove beetles (Coleoptera: Staphylinidae). These polyphagous predators are abundant at the ground level (= epigeal predators) in many temperate agroecosystems. They consume a wide variety of crop pests including aphids (see Snyder and Wise, 1999, for further references), and there is growing evidence that they contribute significantly to a reduction in aphid densities (e.g. Sunderland, 1988; Holland and Thomas, 1997; Sunderland and Samu, 2000; Firlej *et al.*, 2013). Within the vegetation layer, web-making linyphiid spiders also cause high mortalities among aphids, notably in cereals and in orchards (e.g. Sunderland *et al.*, 1986; Wyss *et al.*, 1995, Samu *et al.*, 1996). The abundance and efficiency of generalist predators is increased significantly by a diversification of cropping systems (Wratten and van Emden, 1995; Sunderland and Samu, 2000).

The European earwig *Forficula auricularia* (Dermaptera, Forficulidae) is common and sometimes an important predator of *Eriosoma lanigerum* (woolly apple aphid) in orchards. Earwigs may also reduce aphid numbers in cereal fields (Sunderland, 1988).

## **Aphid Parasitoids**

### **General biology**

Aphids are commonly attacked by hymenopteran parasitoids. With some 50 described genera and over 600 species, the subfamily Aphidiinae (Hymenoptera: Braconidae) is the largest (Mackauer and Starý, 1967). The majority of species are described from the temperate and subtropical zones of the northern hemisphere. Among the Aphelinidae (Hymenoptera), all species in the genus *Aphelinus* and related genera (Starý, 1988) and several species of *Encarsia* (Evans *et al.*, 1995) use aphids as hosts. In addition, several species of gall midge (Diptera: Cecidomyiidae) are parasitic on aphids (e.g. Muratori *et al.*, 2009); with one species, *Endaphis gregaria*, being gregarious (Mackauer and Foottit, 1979). Except for some host records, only scant information is available on the biology of the aphid-parasitic *Encarsia* and *Endaphis* species.

### ***Aphidiinae (Fig. 11.7)***

All species in this subfamily are solitary endoparasitoids (Starý, 1970, 1988). Females normally



**Fig. 11.7.** Aphidiinae. Adult of *Aphidius rhopalosiphi*. Inset: mummy of *Metopolophium dirhodum* parasitized by *Aphidius rhopalosiphi*. (Photographs courtesy of G.R. Gowling.)

deposit a single egg in an aphid, although clutches greater than one or superparasitism may occur when unparasitized hosts are scarce or unavailable (Mackauer, 1990). Supernumerary larvae are eliminated by contest in the early first instar, or by physiological suppression in later instars, so that normally only one larva per host completes development (Chow and Mackauer, 1986; Mackauer, 1990). However, facultative gregarious development was observed in *Ephedrus californicus* (Mackauer and Chow, 2012). After eclosion from the egg, the larva feeds first on the aphid's haemolymph, but later feeds destructively on other tissues, thereby killing the host. There are three larval instars. The development of teratocytes, specialized cells derived from the serosal membrane of the parasitoid embryo, has been well characterized in *Aphidius ervi* (Falabella *et al.*, 2009) and such cells are common in Aphidiinae. Teratocytes process host tissues in ways that greatly improve the efficiency of nutrient transfer from host to parasitoid. The mature larva spins a cocoon either inside (e.g. species of *Aphidius*, *Ephedrus*, *Lysiphlebus*, *Pauesia* and *Trioxys*) or below (species of *Dyscritulus* and *Praon*) the empty host exoskeleton, called a mummy.

Except for some thelytokous species, in which males are rare or absent (Sandrock *et al.*, 2011), the majority of aphidiine wasps are bisexual. Females can determine offspring sex by controlling sperm release; unfertilized and fertilized eggs develop into sons and daughters, respectively (i.e. arrhenotoky).

Because daughters achieve greater fitness benefits from increased size than sons, mothers deposit fertilized eggs selectively in large hosts that are assumed to have higher quality than small hosts (Cloutier *et al.*, 1991). Most females collected in the field are mated (Mackauer, 1976); average offspring sex ratios have a moderate female bias (Singh and Pandey, 1997). Both partial sib mating, local mate competition on the natal patch, and off-patch matings among non-sibs are common features of aphidiine biology (Mackauer and Völkl, 2002; Nyabuga *et al.*, 2012). Sandrock and Vorburger (2011) show that thelytoky in *Lysiphlebus fabarum* is inherited as a single-gene recessive trait. This explains in part why some populations of *L. fabarum* are sexual and some are thelytokous (Engelstädter *et al.*, 2011).

Females mature eggs throughout their reproductive life. Eggs are relatively small and nutrient-poor (Le Ralec, 1991). A female may store between 100 and 400 mature eggs in her ovaries. Egg resorption does not occur in all species (Völkl and Mackauer, 1990; Le Ralec, 1991), but has been documented in sugar-fed *Binodoxys communis* held for more than 48 h without hosts (Dieckhoff and Heimpel, 2010). Lifetime fecundity is potentially very high, ranging between 300 and over 1800 eggs/female under optimal laboratory conditions (Kambhampati and Mackauer, 1989; Hågvar and Hofsvang, 1991), but fecundity is probably much lower in the field, where few adults survive for more than 2–3 days (Mackauer, 1983; Dieckhoff *et al.*, 2014). Adults feed on aphid honeydew and floral or extrafloral nectaries (Tylianakis *et al.*, 2004; Dieckhoff *et al.*, 2014). In temperate climates, diapause is induced by both abiotic (e.g. temperature, photoperiod) and biotic (e.g. host and/or host-plant) signals (Brodeur and McNeil, 1989; Polgár and Hardie, 2000). Although parasitoids typically have higher temperature requirements than their hosts, and hence appear later in the spring (Campbell *et al.*, 1974), the combination of high fecundity, short generation time and female-biased sex ratio results in a high intrinsic rate of increase ( $r_m$ ), ranging between 0.2 and 0.6 females/female/day (Force and Messenger, 1964; Kambhampati and Mackauer, 1989).

### **Aphelinus and related species**

These parasitoids are also solitary. Unlike aphidiine parasitoids, *Aphelinus* females must feed on host haemolymph for egg maturation, often using low-quality host stages for feeding and high-quality

hosts for oviposition (Bai and Mackauer, 1990; Wu and Heimpel, 2007). Females rarely superparasitize (Bai and Mackauer, 1990). When few hosts are available, the relatively large and nutrient-rich eggs are resorbed, thereby prolonging the female's lifespan (Mackauer, 1982). Mature larvae pupate inside the host mummy, which is bluish-black and loosely attached to the substrate.

Aphelinid wasps are relatively small, measuring only 1–2 mm in length, which limits the female's ability to handle large aphids (Gerling *et al.*, 1990). Mated females deposit fertilized and unfertilized eggs differentially in large and small hosts, respectively (Asante and Danthanarayana, 1993); this pattern of offspring sex allocation is independent of the mother's body size (Honěk *et al.*, 1998). Under optimal laboratory conditions, aphelinid wasps may survive for 3–4 weeks and produce between 200 and 800 progeny (Force and Messenger, 1964; Mackauer, 1982; Honěk *et al.*, 1996). However, the number of hosts killed by feeding and oviposition can be much lower, averaging only three aphids per day over 27 days in *Aphelinus flavus* (Hamilton, 1973).

### Foraging behaviour and host finding

Although all parasitoids cause host mortality, the impact on the host population is determined by the parasitoid's reproductive strategy. For example, a female's between- and within-patch foraging behaviour determines the number and type of hosts parasitized in each aphid colony. Therefore, identifying the patterns and processes of foraging behaviour and of the cues involved in host finding and acceptance may help in the planning and implementation of effective biological control programmes.

#### Between-patch foraging behaviour

Aphidiine wasps use a variety of host-plant or host-borne cues to locate aphid colonies (Mackauer *et al.*, 1996). The relative importance of these cues can vary considerably depending on the spatial scale (Völkl, 2000). When foraging between habitats or between host plants (i.e. over relatively long distances so that hosts cannot be found by walking), females often respond to olfactory cues by directed movement. Volatile host-plant compounds serving as semiochemicals can provide the parasitoid with the first information about the potential presence of aphids (Wickremasinghe and van Emden, 1992). For example, Vaughn *et al.* (1996)

showed that females of *Diaeretiella rapae*, a parasitoid of *Brevicoryne brassicae*, responded to volatiles from the aphid's host plant in the Brassicaceae.

A more reliable source than plant-borne cues are aphid-borne ones, although the latter may be less detectable over a long distance. Siphuncular secretions (Grasswitz and Paine, 1992; Battaglia *et al.*, 1993), alarm pheromone (Micha and Wyss, 1996) and sex pheromones (Hardie *et al.*, 1994; Glinwood *et al.*, 1999) are all attractive to aphidiine parasitoids. Herbivore-induced plant volatiles are also used for long-range orientation (Du *et al.*, 1998; Mölck *et al.*, 1999). These cues are particularly important for generalists that parasitize aphids feeding on a variety of plants. Wind tunnel and olfactometer experiments have shown that the searching behaviour of *A. ervi* was influenced by synomones released by broad beans (*Vicia faba*) infested with *Acyrtosiphon pisum* (Du *et al.*, 1998; Powell *et al.*, 1998; Guerrieri *et al.*, 1999). The directed flights of *Aphidius rosae* within a rose bush towards branches infested with *Macrosiphum rosae* was guided by both aphid-borne cues and herbivore-induced volatiles (Völkl, 1994a). However, a parasitoid's response to plant volatiles depends on prior experience (Guerrieri *et al.*, 1997); naïve females of *A. ervi* showed no directed response towards plants infested with pea aphids in a seminatural setting (Schwörer and Völkl, 2001) and naïve *B. communis* females had a much weaker response to host-plant complexes than experienced females (Wyckhuys and Heimpel, 2007).

At the intermediate foraging scale, potential hosts can be found within walking distance or short-range flight, but outside the distance allowing visual host recognition by the parasitoid (Völkl, 1994a). Aphid parasitoids often search systematically (Li *et al.*, 1992) and use gustatory and olfactory cues for rapid host location. Aphid honeydew is an important contact kairomone for both parasitoids (Bouchard and Cloutier, 1984) and hyperparasitoids (Grasswitz, 1998; Buitenhuis *et al.*, 2004). Parasitoids remain longer on a honeydew-contaminated plant, which increases the probability of host location (Cloutier and Baudouin, 1990; Budenberg and Powell, 1992). Aphid alarm pheromone and secretions from the siphuncles also influence searching at the intermediate scale (Grasswitz and Paine, 1992; Battaglia *et al.*, 1993). Some aphidiine species use the presence of honeydew-collecting ants as an indirect indication of potential hosts; female *Pauesia pini*, a parasitoid of *Cinara piceicola*

(green striped spruce aphid) feeding on *Picea abies* (Norway spruce), searched spruce seedlings more intensively after contact with foraging red wood ants (*Formica polyctena*) than after contact with honeydew in the absence of ants (Völkl, 2000). Aphidiine wasps, such as *Lysiphlebus* species, exploiting aphids associated with trophobiotic ants, respond similarly and possibly even use the ants' communication system (e.g. trail pheromones) to locate their hosts (Völkl, 1997).

### **Within-patch foraging behaviour**

For short-range detection, aphid parasitoids use distance-restricted visual and gustatory information to locate and identify suitable hosts. Visual cues, including aphid colour, shape, size and movement, can be evaluated from a short distance without physical contact (Michaud and Mackauer, 1995; Chau and Mackauer, 2000). Contact chemosensory cues (i.e. gustatory cues) located in the aphid's cuticle induce various behavioural responses in aphidiine wasps (Mackauer *et al.*, 1996). In laboratory tests, females of *Aphidius colemani*, *Aphidius picipes* and *Aphelinus abdominalis* responded to aphids at a distance of 3–4 mm, independent of aphid size, but they were unable to discriminate between host and non-host aphids at that distance (Le Ralec *et al.*, 2005).

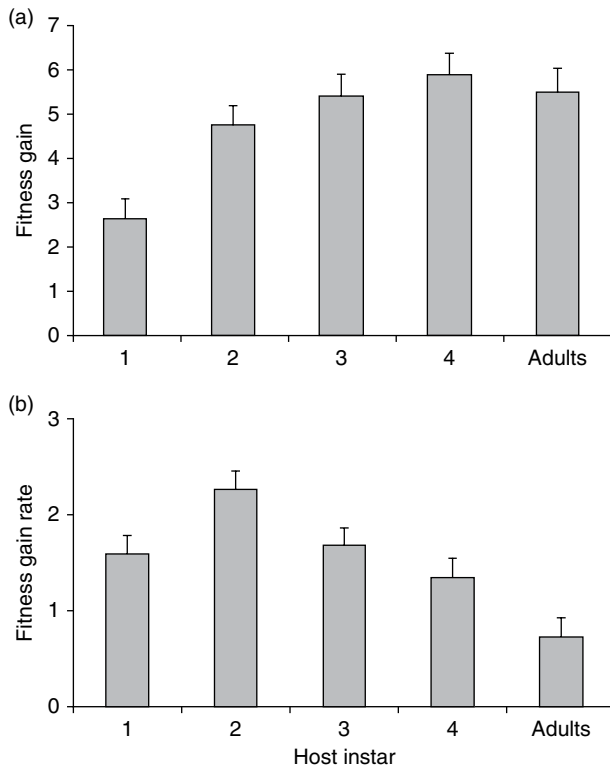
Having successfully located a potential host, the female uses antennation and ovipositor probing to determine whether the host is suitable. Although females of most aphidiine species oviposit in a broad range of host sizes, including aphid embryos (Mackauer and Kambhampati, 1988), they have a preference for particular larval instars and morphs (Chau and Mackauer, 2001; Henry *et al.*, 2005; Wyckhuys *et al.*, 2008). When exploiting an *M. persicae* colony, *A. colemani* females attacked the more profitable hosts (large aphids) initially, but as the patch became depleted, they switched to accepting the less profitable hosts (small aphids) more frequently (Barrette *et al.*, 2010).

Parasitoid preference for host size is correlated with the host's nutritional quality and handling time. Host quality is determined by both species-specific and individual-specific attributes; the latter include aphid size and age at parasitization, attributes that determine an aphid's potential growth until death (Sequeira and Mackauer, 1992). Host quality differs for female and male progeny (Mackauer, 1996). Host quality is an intrinsic property of the host that, presumably, can be evaluated according to absolute

criteria, whereas host value is a relative and dynamic property that varies with parasitoid state variables such as age, egg load and prior experience, as well as with the female's mortality risks (Weisser *et al.*, 1994; Mackauer *et al.*, 1996) and temperature (Moiroux *et al.*, 2015). For example, after several ovipositions, and hence a low egg load, a female may place a low value on a high-quality host (and reject it); by contrast, it may place a high value on a low-quality host (and accept it) if hosts of higher quality are not available (Michaud and Mackauer, 1995). However, egg load did not influence the acceptance of low-quality host species by two *Aphelinus* species (Hopper *et al.*, 2013), which suggests that the ranking of host species in terms of quality may be static rather than dynamic for aphid parasitoids. One potential explanation for an insensitivity in host-choice decisions correlated with egg load is that some aphidiines are able to mature eggs rapidly, thus maintaining a relatively high egg load even after bouts of oviposition (Dieckhoff and Heimpel, 2010; Dieckhoff *et al.*, 2014).

A female can increase offspring fitness by attacking a high-quality, large aphid; however, compared with small aphids, large aphids are able to defend themselves better against attack and can escape (Gerling *et al.*, 1990; Chau and Mackauer, 2001; Wyckhuys *et al.*, 2008). To counter escape by the host, females of *Monoctonus paulensis* hold an aphid with the forelegs for oviposition (Chau and Mackauer, 2000, 2001). Similarly, *Binodoxys* and related genera use abdominal claspers to grasp aphids during oviposition (Desneux *et al.*, 2009). Although offspring developing in large hosts gained in body size and had higher fecundity, females of some species selectively attacked the smallest available aphids, i.e. ones that needed less time for handling and were less likely to escape (Chau and Mackauer, 2001). Barrette *et al.* (2009) showed that handling time was important for the estimation of host profitability when used as a predictor of a parasitoid's choice. In the case of *A. colemani* exploiting *M. persicae*, the fitness gain rate was a better currency for predicting host choice than fitness gain (Fig. 11.8). Late aphid instars were more profitable to *A. colemani* than earlier ones in terms of fitness gain, but they imposed a significant cost because of their increased capacity to defend themselves. These results suggest that parasitoid females assess host profitability from a combination of physiological characteristics and handling time. Other species do show a preference for larger hosts (Lin and Ives, 2003).





**Fig. 11.8.** Host profitability in function of host instar in *Aphidius colemani* parasitizing *Myzus persicae*. (a) Fitness gain per host estimated with life-history traits measurement (development time, sex ratio, potential fecundity); (b) fitness gain rate estimated with life-history traits (development time, sex ratio, potential fecundity) and handling time. (From Barrette *et al.*, 2009.)

Because normally only one offspring per host survives, females generally reject already parasitized aphids when unparasitized ones are available (Mackauer, 1990). Parasitized aphids are marked with a contact pheromone (Chow and Mackauer, 1986; Hofsvang, 1988). Discrimination between self-parasitized, conspecific-parasitized and heterospecific-parasitized aphids (Völkl and Mackauer, 1990) suggests that the marking pheromones vary among parasitoid species, and possibly also among conspecific females. In addition, stinging and the injection of venom may result in subtle changes in the host's defensive behaviour and a transient paralysis. Mackauer and Chow (1990) showed that in *Aphidius smithi* and *E. californicus*, females 'preferred' pseudoparasitized pea aphids over healthy ones. In contrast, females of *Bimodoxys* spp. rejected paralysed hosts, a fact indicating that paralysis might be a possible mechanism for the avoidance of self-superparasitism (Desneux *et al.*, 2009).

The level of exploitation of an aphid colony is influenced by the distance between colonies (Tentelier *et al.*, 2006), the presence of competitors (Le Lann *et al.*, 2011), the size and quality of the colony (Barrette *et al.*, 2010) and the patch experience of parasitoid females (Lanteigne *et al.*, 2015).

### Male foraging behaviour

Few studies have examined foraging behaviour and mate finding by males. Females of aphidiine wasps release a sex pheromone to attract males (McNeil and Brodeur, 1995; Nazzi *et al.*, 1996). In contrast to females, which mate only once, males can copulate several times throughout their life (Singh and Pandey, 1997). The virtual absence of virgin females (which can produce only sons) in field populations of aphidiine wasps suggests that mate finding is very effective (Mackauer, 1976; Mackauer and Völkl, 2002; Nyabuga *et al.*, 2012).

Virgin females of *Aphelinus asychis* produce a trail sex pheromone, which induces searching behaviour in males (Fauvergue *et al.*, 1995).

### Patterns of resource use

Aphid parasitoids show different levels of host specialization. Some species attack a few aphid species, whereas others specialize on habitat and exploit most aphid species within a given habitat (Stilmant *et al.*, 2008; Desneux *et al.*, 2012). Life-table studies indicate that most species of aphid parasitoids can,

at least in theory, overwhelm their host population (e.g. Mackauer, 1983) and hence should make effective biological control agents. In practice, however, average rates of parasitism in the field are often less than 5%, which is usually insufficient for control in most cases (Hughes, 1989). A number of extrinsic and intrinsic factors account for low impact (Mackauer and Völkl, 1993; Boivin *et al.*, 2012). Extrinsic factors, which include environmental conditions and the risk of female mortality (e.g. from predation or hyperparasitism), are discussed first.

Adverse weather conditions such as wind and rain reduce foraging activity, chiefly by preventing females from dispersing in search of hosts (Fink and Völkl, 1995; Weisser *et al.*, 1997; Schwörer and Völkl, 2001). Although a wasp's mean residence time per patch is longer during adverse weather conditions than during optimal conditions, the total number of ovipositions declines, which could explain why many parasitoids have low reproductive success in the field despite their high potential fecundity (Fink and Völkl, 1995; Weisser *et al.*, 1997). Moreover, parasitoids may change their foraging behaviour in response to perceived adult mortality risks (Rosenheim, 1998). For example, females of *Pauesia silvestris*, a parasitoid of *Cinara* species on Scots pine, foraged more thoroughly on the bark (as opposed to needles) in the absence of honeydew-collecting ants. However, in the presence of ants, which capture and kill parasitoids, females searched more intensively on needles, where they were safe from such attacks (Völkl and Kroupa, 1997). Intraguild predation (see below), ants (see below) and hyperparasitism (Sullivan and Völkl, 1999; Brodeur, 2000) can also limit the potential of parasitoids to suppress aphid populations. For example, Brodeur and McNeil (1992) found levels of hyperparasitism exceeding 65% in mummies harbouring *Aphidius nigripes* in potato fields.

Three general patterns of resource use can be distinguished: host resources are exploited well below the female's actual egg load; the degree of resource exploitation varies with the female's egg load; and the degree of resource exploitation varies with the host type. These patterns are not mutually exclusive but overlap to some degree.

#### **Low resource utilization**

Most aphidiine and *Aphelinus* species parasitize a relatively small number of aphids in each colony,

regardless of colony size and egg load (Mackauer and Völkl, 1993). Females of *A. rosae* laid, on average, only 2.8 eggs in each colony of rose aphids comprising 10–40 aphids, although the ovaries of each female contained at least 200 mature eggs. Females were most successful attacking aphids in small and medium-sized colonies, while they quickly abandoned large colonies after only a few ovipositions (Mackauer and Völkl, 1993). Similarly, Ives and Settle (1996) found that *A. ervi* provided with high densities of pea aphid produced very few or zero mummies.

#### **Resource utilization varies with ant attendance**

This phenomenon is typically found in *Lysiphlebus* species. Foraging behaviour of *Lysiphlebus cardui* for *Aphis fabae cirsiacanthoides* on *Cirsium arvense* was influenced by trophobiotic ants (Völkl, 1994b). In the presence of ants, females generally remained in a discovered aphid colony for long periods, often more than one day, until they had depleted the supply of 200–300 mature eggs in their ovaries or had parasitized all available aphids. However, females laid significantly fewer eggs and dispersed earlier in aphid colonies that were not attended by ants. The aphids' defence behaviour was reduced in the presence of guarding ants, which favoured increased oviposition by the parasitoid. Average rates of parasitism in ant-attended colonies increased with season and could reach 100% due to the parasitoids' increasing colonization success (Weisser, 2000). Similar patterns of resource exploitation were observed in other *Lysiphlebus* species.

#### **Resource utilization varies with the host type**

In *P. pini*, patterns of host utilization differed markedly with the generation of its main host, *C. piceicola*, feeding on Norway spruce. Average patch times were longest, and females laid the highest number of eggs when searching for fundatrices and fundatrigeniae in early spring. Oviposition numbers were constrained only by the number of available hosts or eggs, whichever was lower (Völkl and Novak, 1997). By contrast, females laid fewer eggs (independent of colony size) when foraging for viviparous aphids or oviparae later in the season. Rates of parasitism and the number of mummies per colony were lowest during midsummer, when *P. pini* exploited alternate hosts (e.g. *Cinara pinea*) on pine. This seasonal pattern of parasitization was correlated with variation in host size. Differences

between years were due mainly to variations in environmental conditions. Low temperatures in spring favoured *P. pini*, while high temperatures led to decreased parasitism rates; this could be explained by differences between the activity patterns of *P. pini* and worker ants (*F. polyctena*) that protected the aphids. Parasitoid females were very active at low temperatures and showed increased oviposition rates, while the activity of the worker ants was reduced drastically. A similar pattern of parasitism was observed in *Euaphidius cingulatus*, a parasitoid of aphids in the genus *Pterocomma* on willows (Völkl, 1997). Laboratory studies have also shown that the defensive behaviours of alate aphids and alate nymphs were more effective than those of apterae of similar size (Wyckhuys *et al.*, 2008).

### Aphid Pathogens (Fig. 11.9)

The most significant microbial natural enemies of aphids all belong to the Fungi, although a few RNA viruses have been recorded (van den Heuvel *et al.*, 1997; Moon *et al.*, 1998; van Munster *et al.*, 2002). Most fungi infecting aphids are found in the Entomophthoromycota or the Ascomycota, although



**Fig. 11.9.** *Acyrthosiphon pisum* infected by the entomopathogenic fungus *Pandora neoaphidis*. (Photograph courtesy of Rothamsted Research, Harpenden, UK.) The insert shows the distance over which fungal spores from the cadaver are projected. (Photograph courtesy of J.K. Pell.)

species that contribute most regularly to the regulation of aphid populations are from the Entomophthoromycota (Pell *et al.*, 2001). At least 29 species of Entomophthoromycota are known to infect aphids (Keller, 2006). Among these, *Pandora neoaphidis*, an aphid specialist infecting >70 species, occurs most frequently, has a worldwide distribution and regularly causes epizootics (Pell *et al.*, 2001; Nielsen *et al.*, 2003). *Neozygites fresenii*, *Conidiobolus obscurus*, *Zoophthora radicans*, *Entomophthora planchoniana*, and occasionally other species, can also be locally abundant (Pell *et al.*, 2001; Steinkraus, 2006). Our knowledge about how common particular species are is influenced both by their ecological traits and by the timing and location of research conducted on them.

Entomophthoroid species are usually obligate pathogens, while aphid-pathogenic ascomycete species can be facultative. Ascomycota infecting aphids in the field often belong to the genera *Beauveria*, *Isaria* (= *Paecilomyces*), *Lecanicillium* (= *Verticillium*) or *Metarhizium* (Steinkraus, 2006). *Lecanicillium* species from the *lecanii* species complex are the only ascomycetes that regularly cause aphid mortality in nature (Milner, 1997); occasionally they cause epizootics in aphid populations in tropical and subtropical areas (Shah and Pell, 2003).

### General biology of aphid-pathogenic fungi

Conidia (spores) are responsible for infection during the season when aphids are most active. After the death of an infected aphid, fungi exit from within the cadaver, particularly through the intersegmental membranes (Butt *et al.*, 1990). Many thousands of conidia are then produced externally from each aphid cadaver, with numbers depending on aphid biomass (Steinkraus *et al.*, 1993).

Conidia of some species are hydrophilic, being covered with pre-formed mucous (e.g. *Lecanicillium* and entomophthoroid species), while the surfaces of *Beauveria* and *Metarhizium* conidia are hydrophobic (Boucias and Pendland, 1991). Both types of conidia adhere to host cuticle non-specifically and can germinate rapidly; penetration through the cuticle is accomplished by both enzymatic and mechanical means (Askary *et al.*, 1999). If an aphid is moulting at the time of cuticular penetration, infection can be prevented because the invading fungus is shed with the cast skin (Kim and Roberts, 2012). Once the fungus has penetrated the cuticle, it grows either as wall-less protoplasts (some

entomophthoroid species), hyphal bodies, or blastospores within the aphid. Infected aphids produce fewer progeny before death than uninfected ones (e.g. Fournier and Brodeur, 2000; Baverstock *et al.*, 2006). For entomophthoroid species, host death is usually caused by physiological starvation of the host after the fungus has utilized all internal contents. For ascomycetes, dying hosts often contain only sparse vegetative growth, and fungal growth continues after the host is dead (Vega *et al.*, 2012). For many aphid/fungus combinations, death occurs approximately 3–5 days after infection at 20°C, but can vary with temperature (e.g. Baverstock *et al.*, 2008a) and the initial concentration of inoculum (e.g. Fournier and Brodeur, 2000; Hesketh *et al.*, 2008).

Some fungal species influence the behaviour of their hosts prior to death, often to improve the likelihood of transmission (Roy *et al.*, 2006; Hughes *et al.*, 2012). Pea aphids responded less to alarm pheromone prior to death when infected with *P. neoaphidis*; this change in behaviour favours the fungus, as the preservation of aphids in close proximity to one another would improve transmission. Infection of pea aphids with either *P. neoaphidis* or *Beauveria bassiana* also results in higher proportions of progeny developing into alatae (Hatano *et al.*, 2012); although this could have evolved to aid aphid escape from infection, it may also facilitate pathogen dispersal.

Host specificity is a highly variable biological trait among species of entomopathogenic fungi. A generalist species may infect and develop successfully in hosts of numerous taxa, while a specialist would infect only one, or a few, host species (Brodeur, 2012). The host range of entomophthoroid species is generally narrower (see below), while for ascomycete species the host range is broader. However, in recent years, molecular analyses of commonly occurring entomopathogenic ascomycetes have shown that what was previously considered as one species could, in fact, be several species. For example, *B. bassiana sensu lato* (Rehner and Buckley, 2005), *Metarhizium anisopliae sensu lato* (Bischoff *et al.*, 2009) and *Lecanicillium lecanii sensu lato* (Zare and Gams, 2001) are each composed of several species. Since these taxonomic changes have been made, the host specificity of the newer species has not been entirely determined and, in many cases, the identities of isolates evaluated in older publications on host specificity are simply not known. However, it appears that what can now be

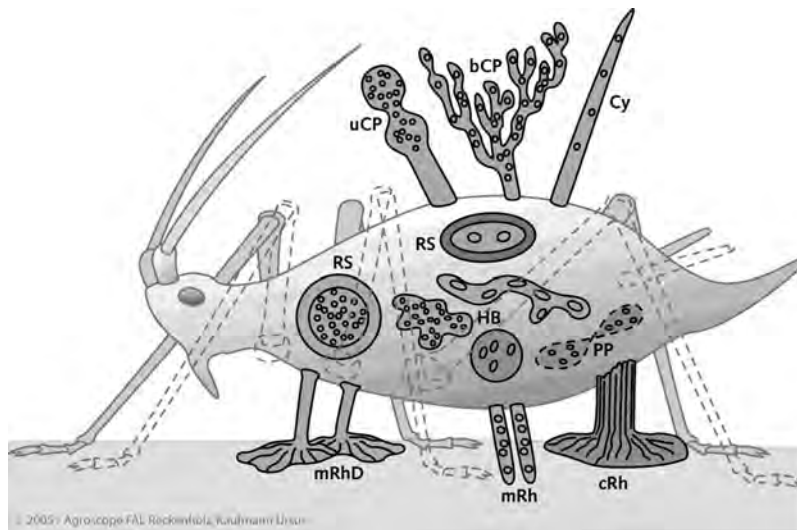
identified as *B. bassiana sensu stricto* should still be considered as an opportunist capable of exploiting insects from numerous taxa (Rehner and Buckley, 2005).

### Specialized biologies of entomophthoroid species

Entomophthoroid species have unique attributes that are not shared with the distantly related ascomycetes. First, conidia produced by entomophthoroid species are typically actively discharged from conidiophores on cadavers by hydrostatic pressure (Pell *et al.*, 2001). Conidial discharge continues for some time; for *Pandora nouryi* emerging from *M. persicae* cadavers under high humidity, peak conidial discharge occurred 12–20 h after aphid death at >15°C (Li *et al.*, 2006). After host death, cadavers are often fixed in place on the plant by fungal rhizoids that emerge through the ventral surface of the cadaver (e.g. *P. neoaphidis*, *Zoophthora* species) or through the proboscis (e.g. *C. obscurus*) (Fig. 11.10). Attachment to the substrate ensures that the fungus remains in an optimal environment (often elevated or near other hosts) to promote transmission (Roy *et al.*, 2006).

If primary conidia land on non-host surfaces, they produce another conidium (a secondary conidium) that, in *P. neoaphidis*, is also actively discharged, thus increasing the ability of the fungus to reach and infect new aphid hosts. In *Zoophthora* and *Neozygites* species, higher-order conidia are not always actively discharged but are produced on fine capillaries called capilliconidiophores. The capilliconidium is borne on the top of the conidiophore some distance above the surface and remains attached until a host dislodges it. In *Zoophthora* species, both primary and secondary conidia are infective (Glare *et al.*, 1985; Pell *et al.*, 1993). In *N. fresenii*, only the secondary capilliconidia are infective and the primary conidia serve only as a method of dispersal (Steinkraus, 2006).

Most entomophthoroid species also form persistent double-walled resting spores that are sexually (zygospores) or asexually (azygospores) produced within cadavers as a mechanism for survival during periods when aphids are not present or are at small population densities (Bitton *et al.*, 1979). Dead infected aphids containing resting spores remain on foliage and bark or fall to the ground, where the spores can remain dormant for many years. Laboratory studies with *P. nouryi* in *M. persicae*



**Fig. 11.10.** Entomophthorean structures present inside infected aphids and on the aphid surface. Projected conidia (asexual spores) are not shown. PP = protoplasts; HB = hyphal bodies; RS = resting spores (zygospores or azygospores); uCP = unbranched conidiophores (specialized hyphae from which conidia are produced); bCP = branched conidiophores; Cy = cystidia; mRhD = monohyphal rhizoids with disc-like (specialized) holdfast; mRh = monohyphal rhizoids without specialized holdfast; cRh = compound rhizoid. (From Keller, 2006.)

demonstrated that between 15 and 25°C, resting spore production was associated positively with inoculum level and associated secondarily with temperature and photoperiod (Zhou and Feng, 2010). However, winter field studies in the same system, when temperatures were often <10°C, found that low temperatures were the most important variable associated with resting spore formation (Zhou *et al.*, 2012). Resting spores germinate by producing forcibly discharged germ conidia or capilliconidia (the latter only in *N. fresenii*), both of which are infective. Resting spores are formed by *P. neoaphidis* in Argentina (Scorsetti *et al.*, 2012), although this fungal species is not known to make resting spores in the northern hemisphere. There, overwintering forms reported include dormant ‘loricoconidia’ in the soil (Nielsen *et al.*, 2003) and specialized spherical hyphal bodies within cadavers on aboveground plant substrates where relative humidity is low (Feng *et al.*, 1992). In the laboratory, cadavers can remain capable of producing infective conidia for circa 80–96 days when maintained at a constant 5°C on moist soil, although this capacity is reduced to less than 50 days under natural winter conditions (Nielsen *et al.*, 2003; Baverstock *et al.*, 2008a). In areas where aphids are present during winter, this ability to persist during colder periods could allow

cycles of re-infection to occur during winter (Feng *et al.*, 1991). Specialized persistent hyphal bodies within winter-collected aphid cadavers have also been observed for *E. planchoniana* (Keller, 1987).

### Epizootiology of entomophthoroid fungi

Epizootiology is ‘the study of animal disease dynamics on the basis of mass phenomena’, and therefore deals with disease on a population level (Shapiro-Ilan *et al.*, 2012). Although the basic fungal life cycle is relatively simple, the epizootiology of fungi within populations is complex and regulated by the host population (density, susceptibility, distribution), the fungus population (density, virulence, distribution) and the environment (temperature and humidity), all of which are linked by transmission (Steinkraus, 2006). Both aphid and fungus populations are discontinuous in the environment, so evolution of the mechanisms that encourage transmission within and between host populations is essential for fungal survival.

### Abiotic conditions

Fungi cannot germinate, infect or sporulate if ambient relative humidity falls below 90–93%, making

moisture the most important limiting factor (Millstein *et al.*, 1983; Hemmati *et al.*, 2001). *Pandora neoaphidis* infected cadavers produce more conidia when in direct contact with water than when in a saturated environment (Wilding, 1969). There is a positive correlation between *N. fresenii* and *E. planchoniana* induced aphid mortality and the preceding hours of leaf wetness (Ekblom and Pickering, 1990); high infection levels are also associated with rainfall (Hemmati *et al.*, 2001). Ambient humidity may be essential in the initiation of an epizootic, but thereafter it becomes secondary as, during the night at least, microclimatic conditions are usually favourable (Hemmati *et al.*, 2001). Both *P. neoaphidis* and *Z. radicans* have evolved an internal clock set by the time of dawn that regulates the timing of host death such that it occurs at the end of the photophase, thereby ensuring that sporulation and infection occur during the night (Milner *et al.*, 1984). *Neozygites fresenii* also sporulates most profusely at night, suggesting a similar mechanism (Steinkraus *et al.*, 1996, 1999).

Temperature affects the number of conidia produced by an aphid cadaver and the rate at which sporulation and infection (time to death) occur; these in turn affect the rate of epizootic establishment. Suboptimal temperatures reduce the number of conidia produced from *Z. radicans* and *P. neoaphidis* infected cadavers and extend the time to germinate, infect and kill (e.g. Leite *et al.*, 1996), thereby reducing the rate of transmission and epizootic development. While many entomophthoroid species are less active as temperatures increase, *N. fresenii* is well adapted to function in hot weather (Steinkraus *et al.*, 1991, 1995).

### **Dispersal and contact rate**

Actively discharged conidia escape the boundary layer and enter the airstream, facilitating local and distant dispersal (Steinkraus, 2006). Conidia of *N. fresenii* and *P. neoaphidis* have been detected in the air above, and some distance from, crops infested with infected aphids (Steinkraus *et al.*, 1996, 1999; Hemmati *et al.*, 2001). This is an important mechanism for dispersal between patchily distributed hosts and is highly effective when large numbers of aphids are present. However, it relies entirely on wind direction and many conidia may be lost.

Dispersal of fungi within infected and migrating alatae is more targeted. In a series of studies in China, 19–37% of dispersing alatae from nine different

species that landed on isolated, elevated sentinel plants (wheat, brassicas, lettuce) were infected with fungi from five entomophthoroid species (predominantly *P. neoaphidis*) (Feng and Chen, 2002; Huang *et al.*, 2008). Furthermore, in flight mill experiments, *P. neoaphidis* infected aphids flew further than uninfected aphids and, having landed, established colonies that subsequently became infected, demonstrating the potential for dispersing alatae to establish infection in a new location (Chen and Feng, 2006).

Another targeted form of dispersal is through vectoring by co-occurring insect natural enemies, particularly predators that have been contaminated by conidia while foraging and then carry the inoculum to new aphid populations. Transmission of *P. neoaphidis* by the ladybirds, *C. septempunctata* and *Harmonia axyridis* (harlequin ladybird) has been reported (Pell *et al.*, 1997; Roy *et al.*, 2001; Wells *et al.*, 2011).

Once an inoculum has entered an aphid population, the rate of transmission is modulated by the rate of contact with new hosts. This can be influenced by both host and inoculum density and distribution, but also by host mobility. In response to insect natural enemies, aphids move around more in order to escape, and this increases their likelihood of contacting inoculum; foraging by both predators and parasitoids increases the transmission of *P. neoaphidis* in surviving populations of several aphid species (Roy *et al.*, 1998; Baverstock *et al.*, 2009). Even the presence of co-occurring herbivores can encourage aphid movement as a response to crowding, thereby increasing transmission (Baverstock *et al.*, 2008b). In a mesocosm-scale experiment, transmission of *P. neoaphidis* in aphid populations on patches of plants of the same species (a host of the aphid) was greater than in aphid populations on patches of plants with a mixture of species (including non-hosts of the aphid) (Baverstock *et al.*, 2012). The mechanism for this was related to the mobility and resulting density of the aphids and not the plants themselves; aphids in the single plant species patch distributed themselves evenly among plants at low densities, while in the multiple plant species patches, aphids became concentrated on a limited number of suitable host plants and transmission was greater.

Although some predators also consume, or partially consume, *P. neoaphidis* infected cadavers, this reduction in overall inoculum levels is small by comparison with the large increases in transmission

observed in the presence of foraging insect natural enemies (Pell *et al.*, 1997; Roy *et al.*, 1998, 2008). However, ants with a mutualistic relationship with aphids rapidly remove *P. neoaphidis* infected cadavers, and also living infected aphids, before sporulation occurs, which could reduce transmission significantly (Nielsen *et al.*, 2010).

### **Host range, resistance and susceptibility**

The host ranges of entomophthoroid fungi are relatively limited. *Pandora neoaphidis*, *E. planchoniana* and *N. fresenii* only infect aphids (Nielsen *et al.*, 2001; Pell *et al.*, 2001). *Zoophthora radicans* has been recorded from numerous different insect orders, although isolates are often more infective to the host species from which they were isolated than to other species (Pell *et al.*, 1993). Within a taxon of insect hosts such as aphids, it can be essential for the survival of the fungus for it to be able to infect a number of different species; *P. neoaphidis* has been recorded from numerous pest and non-pest aphid species (e.g. Pell *et al.*, 2001; Manfrino *et al.*, 2013). Studies comparing the susceptibility of seven pest aphid species to different isolates of *P. neoaphidis* suggest that some aphid species, particularly *Rhopalosiphum padi*, are resistant (Shah *et al.*, 2004), although others have recorded infection of *R. padi* in the field, and sometimes at high levels (e.g. Basky and Hopper, 2000).

The aphid species that is generally considered to be most susceptible to a wide range of *P. neoaphidis* isolates is *A. pisum* (Shah *et al.*, 2004), although great variability in susceptibility has also been demonstrated between alatae and apterae (e.g. Lizen *et al.*, 1985). In particular, greater resistance to *P. neoaphidis* is seen in some races of *A. pisum* specialized to particular leguminous host plants, and is associated with the presence of mutualist endosymbionts (e.g. Ferrari *et al.*, 2004; Scarborough *et al.*, 2005). It would seem that this relationship is highly co-evolved in relation to specialist aphid pathogens; while a symbiont confers resistance in the pea aphid to the specialist pathogen *Zoophthora occidentalis*, it provides no resistance to the generalist *B. bassiana* (Parker *et al.*, 2013). Interestingly, similar relationships with endosymbionts have not been found in the soybean aphid, *Aphis glycines* (Wulff *et al.*, 2013).

For a single clone of pea aphid and a single isolate of *P. neoaphidis*, susceptibility can also vary directly in response to the host plant. Aphids on

pea varieties with low levels of leaf wax were more susceptible to *P. neoaphidis* than individuals on high wax varieties (Duetting *et al.*, 2003), and aphids cultured on beans were more susceptible if returned to beans after inoculation than if they were returned to other leguminous species (Tkaczuk *et al.*, 2007). Among isolates, there is also huge variation in virulence to any particular aphid species (Shah *et al.*, 2004). The epizootiology of diseases caused by different isolates therefore depends on a number of virulence parameters associated with the fungal isolate and susceptibility parameters of the host that could interact in complex ways.

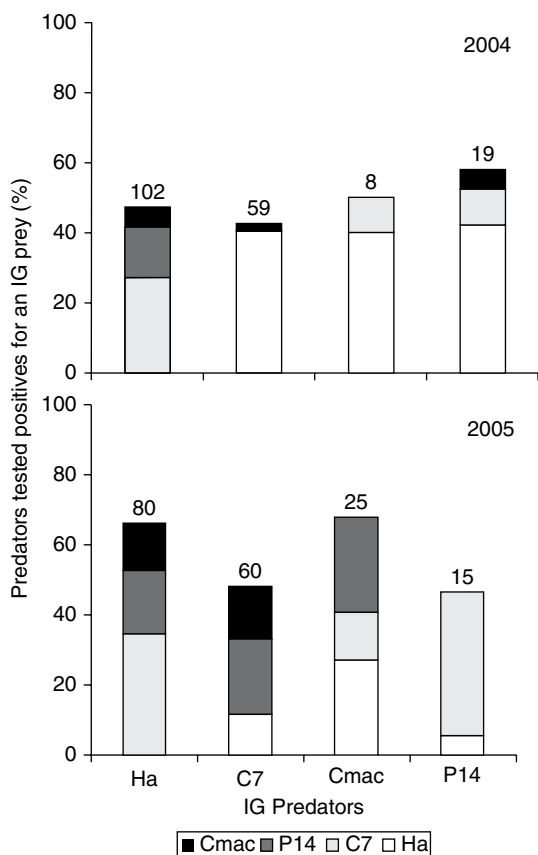
## **Intraguild Interactions and Mutualistic Ants**

### **Intraguild predation and competition**

Natural enemies of aphids exist in natural and managed ecosystems as a community that typically includes taxonomically and functionally different groups: predators, parasitoids and pathogens. These communities can be complex, and species can interact with one another through predation and competition. One form of omnivory is intraguild predation (IGP), where one species of natural enemy attacks another species with which it also competes for a shared prey or host species. For example, multifaceted relationships can evolve between a predator, a parasitoid and a fungus that all exploit the same aphid species. Rosenheim *et al.* (1995) concluded that intraguild interactions were widespread within communities of biological control agents of arthropod pests, and that they were likely to influence the efficacy of biological control. The literature is now filled with evidence of positive, negative, or neutral interactions between different types of biological control agents.

Aphid predators are commonly involved in IGP interactions when competing for the same aphid prey within a colony (Lucas, 2005; Hautier *et al.*, 2008). Field evidence of a high prevalence of IGP among Aphidophaga species comes from the work of Gagnon *et al.* (2011a,b), who used molecular analyses of gut contents to examine the nature and incidence of IGP among four species of coccinellid predators in soybean fields: *H. axyridis*, *C. septempunctata*, *Coleomegilla maculata* (12-spot ladybird) and *Propylea quatuordecimpunctata* (14-spot ladybird). Levels of IGP were very high, with averages of 47% and 59% of all coccinellids containing

DNA of other coccinellids in their guts in 2004 and 2005, respectively (Fig. 11.11). Moreover, 13% of the sampled predators contained two, and even three, other coccinellid species in their gut. Under laboratory conditions, Lucas *et al.* (1998) showed that interactions between the larvae of the lacewing *Chrysoperla rufilabris* and the ladybird beetle, *C. maculata*, resulted in reciprocal predation, i.e. both predators were at risk of being eaten by a competitor. Hindayana *et al.* (2001) found that larvae of the hover fly species *E. balteatus* were



**Fig. 11.11.** Levels of intraguilid predation among four species of ladybird beetles measured by molecular gut content analysis in soybean fields in Québec, Canada, in 2004 and 2005. Results are expressed as the proportion of each species of intraguilid prey detected in the gut of intraguilid predators. Ha = *Harmonia axyridis*; C7 = *Coccinella septempunctata*; Cmac = *Coleomegilla maculata*; P14 = *Propylea quatuordecimpunctata*. Numbers above histogram bars represent the number of individuals tested. (From Gagnon *et al.*, 2011a.)

susceptible to predation by larvae of the lacewing *C. carnea* and the ladybird *C. septempunctata*, while *E. balteatus* pupae were consumed only by lacewing larvae. Lucas *et al.* (1998) suggested that in interactions between members of the same guild, specialist species were more likely than generalists to become prey. In contrast, competition between larvae of the predatory midge *A. aphidimyza* and other aphid predators was always asymmetrical in that gall midge larvae generally served as prey. This species is not adapted to kill prey other than aphids, due to its highly specific feeding requirements (Lucas *et al.*, 1998).

The nature, intensity and outcome of intraguilid predation among aphidophagous predators are influenced by, for example, climatic conditions (Sentis *et al.*, 2014), identity and age structure of intraguilid predators (Lucas *et al.*, 1997; Lucas, 2005), distribution and density of the aphid prey (Lucas and Rosenheim, 2011; Sentis *et al.*, 2013, 2014; Gagnon and Brodeur, 2014) and host-plant architecture (Lucas and Brodeur, 1999).

Aphid parasitoids also experience mortality due to intraguilid predation (Brodeur and Rosenheim, 2000). Brodeur and McNeil (1992) showed that predation of *A. nigripes* mummies on potato accounted for 15% of total parasitoid mortality. Working in cotton, Colfer and Rosenheim (2000) demonstrated that adult coccinellids consumed numerous aphid mummies, although they preferred living aphids as prey. Similarly, *B. communis* in *A. glycines* (soybean aphid) mummies on soybean are at risk of predation, and IGP tends to increase over the season as aphid density increases and more predators are attracted to aphid aggregations (Heimpel *et al.*, 2010). Some species of aphid parasitoid have evolved mechanisms to reduce IGP. For example, *A. ervi* females avoided leaves visited by the larvae and adults of *C. septempunctata* during the previous 24 h, but not if the interval was greater (Nakashima *et al.*, 2004). In contrast, avoidance behaviour was not observed in *L. fabarum* foraging in aphid colonies in which adult *C. septempunctata* and larvae of *E. balteatus* were present (Meyhöfer and Klug, 2002).

Fungal pathogens with a broad host range can also be involved in intraguilid interactions. This applies in particular to some mitosporic fungi developed as mycoinsecticides for use against aphids. For example, *B. bassiana*, *Paecilomyces fumosoroseus* and *Lecanicillium* spp. can infect aphid predators and parasitoids, but often only at high doses and/or



under laboratory conditions favouring the fungus and/or stressing the host (Lacey *et al.*, 1997; Askary and Brodeur, 1999; Pell and Vandenberg, 2002). Physiological susceptibility under laboratory conditions often represents the 'worst case scenario' for infection of beneficial insects, which may differ from ecological susceptibility in the field. Beneficial arthropods and mitosporic mycoinsecticides can, therefore, coexist even when the potential of some isolates and species for infecting beneficial organisms has been demonstrated in the laboratory (James *et al.*, 1998).

Within the guild of aphid natural enemies, arthropod predators can be intraguild predators of fungi, and also affect fungal dispersal and transmission (Roy and Pell, 2000). For example, the ladybird, *C. septempunctata*, and the carabid beetle, *Pterostichus madidus*, both feed on aphid cadavers sporulating with *P. neoaphidis*, in contrast to larvae of the lacewing, *C. carnea*, and the hover fly, *E. balteatus*, which do not consume *P. neoaphidis* infected aphids (Roy *et al.*, 2001). In this study, *P. neoaphidis* infected aphid cadavers were less palatable to *C. septempunctata* larvae than uninfected ones. Hungry larvae did attempt to consume cadavers, but never consumed them entirely. Rates of transmission were increased significantly in the presence of foraging ladybirds (Roy *et al.*, 2001), which suggests that predators may, in fact, enhance the transmission and dispersal of entomopathogenic fungi rather than having a negative impact as intraguild predators (Roy *et al.*, 2001; Pell and Vandenberg, 2002).

Interactions between parasitoids and pathogens are generally asymmetrical in favour of the pathogen. The pathogen invariably kills the host faster than the parasitoid can complete its development, although the final outcome of such competitive interactions is dependent on the relative timing of parasitism and infection (Kim *et al.*, 2005). Development of the parasitoid *A. nigripes* was impeded by the fungus *Lecanicillium* sp., but many parasitoids still emerged from aphids infected 4 days after parasitization (Askary and Brodeur, 1999). Interactions between pathogens and parasitoids are also affected by plant resistance. Fuentes-Contreras *et al.* (1998) reported that aphids and parasitoids developed more slowly on aphid-resistant wheat cultivars, but the time required for an aphid to be killed by a fungus remained the same. Some parasitoids avoid infected hosts (Brobyn *et al.*, 1988). There is some evidence that the combination of parasitoids and fungal pathogens can have an

additive effect with respect to aphid control (Hagen and van den Bosch, 1968); that is, that parasitoids are not affected negatively by entomopathogenic fungi. This is supported by field experiments. For example, recent evidence indicates that aphid control can be enhanced by the combination of different natural enemies. For instance, the combination of the parasitoid *A. asychis* and the fungus *P. fumosoroseus* resulted in better control of *D. noxia* than did the application of a single antagonist; percentage parasitism and parasitoid emergence were not affected by the fungus (Mesquita *et al.*, 1997).

### The effect of mutualistic interactions with ants on predation and parasitism

Many aphid species have evolved mutualistic relationships with honeydew-collecting ants (Buckley, 1987). Both partners derive benefits from this association; the ants gain access to nutrients and, by attacking intruders into an aphid colony (potential competitors for the carbohydrate source), act as effective guards against the aphids' natural enemies. The adults and larvae of coccinellid species were more abundant in areas where ants were naturally absent or experimentally excluded (Sloggett and Majerus, 2000b; Majerus *et al.*, 2007). There is less information about the influence of ants on the density of other predatory species, especially syrphid and chrysopid larvae. Müller and Godfray (1999) and Fischer *et al.* (2001) demonstrated a general decrease in predator density in ant-attended colonies, which in turn persisted longer. On tansy, the predacious bug *A. nemorum* was significantly less abundant in ant-attended colonies of *Metopeurum fuscoviride* than in unattended ones, where the presence of predators significantly reduced colony persistence (Fischer *et al.*, 2001).

Ant-attendance also reduces the risk of parasitism. Völkl (1997) reported that in central Europe, 14 of 40 parasitoid species attacking ant-attended aphids, when approaching an aphid colony, were generally treated aggressively by honeydew-collecting ant workers. Therefore, resource utilization by these parasitoid species was widely restricted to unattended aphid colonies. Foraging parasitoids were more effective when ant-tended aphid colonies were feeding on plants with a high structural diversity (e.g. leaves and flowers of *Chenopodium* spp.) than on plants with a low structural diversity (e.g. stems of the thistle *C. arvense*; Mackauer and Völkl, 1993; Völkl, 1997). A number of predator and

parasitoid species have evolved morphological, chemical and/or behavioural adaptations to gain access to ant-attended resources (e.g. Eisner *et al.*, 1978; Liepert and Dettner, 1993; Völkl and Mackauer, 1993; Sloggett *et al.*, 1998).

Ant-parasitoid and ant-predator interactions can affect the outcome of biological control. An example of this is the release of the polyphagous parasitoid *A. colemani* in the South Pacific for biological control of *Pentalonia nigronervosa* (banana aphid) (Völkl *et al.*, 1990). Small aphid colonies occur mainly in concealed areas of the banana plant, such as the space between pseudostems and leaf sheaths. *Aphidius colemani* females foraged intensively in these areas, but were ineffective as control agents because they were heavily attacked by honeydew-collecting ants (Stechmann *et al.*, 1996). Moreover, the abundance of predators was also reduced by ant attendance (Stechmann and Völkl, 1990).

### The role of defensive aphid endosymbionts

Not all aphids are equally susceptible to parasitism and infection. Even within the same aphid species, considerable variation can exist for resistance to a specific parasitoid (Henter and Via, 1995). Aphids harbour several bacterial endosymbionts. These include the primary (obligate) bacterium *Buchnera aphidicola*, as well as a number of secondary symbionts that are facultative and thus not needed for aphid survival (Douglas, 1998; Oliver *et al.*, 2010). Among the secondary endosymbionts, bacteria have been identified that have a variety of effects on their aphid hosts, including thermal tolerance, efficiency of host-plant use and defence against fungal pathogens and parasitoids (Oliver *et al.*, 2010).

Symbiont-mediated defence has been identified against both parasitoids and pathogens (Oliver *et al.*, 2014). The best-studied symbiont–host–enemy system in this context is *Candidatus* Hamiltonella defensa protecting the pea aphid, *A. pisum*, from the aphidiine parasitoid *A. ervi* (Oliver *et al.*, 2010). This protection (though usually not complete) is mediated by a bacteriophage (‘APSE’) that encodes a toxin acting on parasitoid eggs and/or larvae (Oliver *et al.*, 2010). *Candidatus* H. defensa also protects the black bean aphid, *A. fabae*, against the aphidiine *L. fabarum*, and here variation in defensive performance suggests a co-evolutionary arms race between the symbiont (and/or its phage) and the parasitoid (Schmid *et al.*, 2012; Rouchet and Vorburger, 2014). Last, *Ca. H. defensa* has been

found to protect the cowpea aphid, *Aphis craccivora*, against successful parasitism by two species of *Binodoxys* but not against *Lysiphlebus orientalis* or *A. colemani* (Asplen *et al.*, 2014). This study indicates that the effect of *Ca. H. defensa* and/or its phage may be host-genus specific. However, Hopper *et al.* (unpublished) found that *A. craccivora* was not protected by *Ca. H. defensa* against parasitism by two species of *Aphelinus*. Some endosymbionts other than *Ca. H. defensa* may also confer resistance to aphids, including *Candidatus* Regiella insecticola (Vorburger *et al.*, 2010) and *Spiroplasma symbiotica* (Oliver *et al.*, 2003). Even when effective against some parasitoids, defensive symbionts in aphids are not ubiquitous in aphid populations and frequently occur at intermediate frequencies (Oliver *et al.*, 2014). This is likely to be due to costs of infection and incomplete vertical transmission of the symbionts (Dykstra *et al.*, 2014; Oliver *et al.*, 2014).

Endosymbiont-mediated protection against fungal pathogens has not been studied as well as protection against parasitoids. Bacterial symbionts in the genera *Regiella*, *Rickettsia*, *Rickettsiella* and *Spiroplasma*, which are all distantly related to each other, all protect pea aphids against *P. neophidis* by increasing resistance and lowering transmission (Scarborough *et al.*, 2005; Lukasik *et al.*, 2013). In contrast, the secondary bacterial endosymbiont in soybean aphids, *Arsenophonus*, did not influence infection by *P. neophidis* (Wulff *et al.*, 2013).

### Conclusion

Aphids are attacked by a wide range of natural enemies, many of which are highly fecund and, at least in theory, should be capable of reducing aphid populations below economic thresholds. However, Aphidophaga often fail to achieve their expected potential in the field. Many predators are not very effective in locating aphid prey, and specific habitat requirements (such as the availability of a pollen or nectar supply as food for adults) additionally reduce their activity in the vicinity of aphid colonies. Although parasitoids apparently are very successful foragers within their habitat, species-specific oviposition strategies may cause their departure before all available hosts in a colony are exploited. Similarly, aphid-pathogenic fungi have effective transmission and dispersal mechanisms but can be limited by their requirement for high humidity. Intraguild predation additionally may lower the impact of Aphidophaga.

Biological control of aphid pests has achieved notable successes (Chapter 20, this volume), although the results have remained difficult to predict. We suggest that the practice of biological control currently does not reflect all the available information on predators, parasitoids and pathogens. Such information not only is critically important in the selection of the most appropriate control strategy but also it may explain why control is only partially successful in some situations or in only part of the pest's range.

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# The Ethology of Domestic Animals

## An Introductory Text

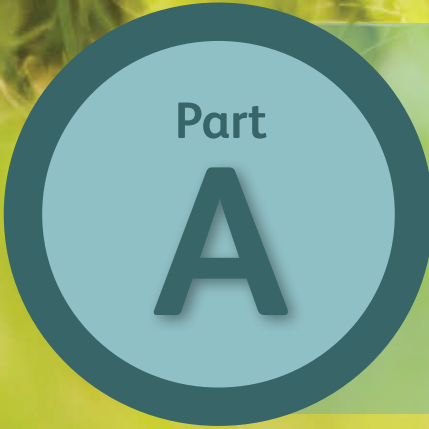
Edited by Per Jensen

3RD EDITION



 **CABI**





# Basic Elements of Animal Behaviour

## Editor's Introduction

The first nine chapters of this book introduce the basic concepts and the central subject matters required for a firm understanding of the biological bases of animal behaviour. The first chapter provides a historical background, which may help in understanding the questions that occupy contemporary ethology and its applied branches. In the second chapter, we approach the important question of how behaviour is controlled by genes (the nature–nurture problem) and also what this means for behavioural evolution. This chapter also describes the process of domestication, which is essential for understanding present-day domestic animals. The third chapter goes to some depth in describing how observable behaviour is a result of physiological processes throughout the body. The concept of motivation has a long history in ethology, and has proven to be essential for understanding the cognitive capacities and needs of animals in captivity, and the concept receives a detailed treatment in the fourth chapter. This leads naturally on to the fifth chapter, where learning and cognition are in focus. Here, considerable scientific advance has been made over the last decade, and this is introduced to the reader together with its relevance for animal welfare.

In Chapter 6, we move towards a more evolutionary and ecological approach to behaviour. Social and reproductive behaviour are important elements of applied ethology, since animals are normally kept in groups and they are expected to reproduce. The topic of the seventh chapter is play behaviour, a phenomenon well-known to all, but until recently poorly understood by science. In Chapter 8, the reader receives a thorough introduction to the concepts of individual variation and personality, of high relevance for ethology and animal welfare. Chapter 9 moves into the important aspects of abnormal behaviour and stress, and raises some central topics in contemporary applied ethology, such as how the behaviour of an animal can be used to assess its welfare and whether it is under stress. Furthermore, the relatively novel insights into positive emotions and welfare are covered. In the tenth and last chapter of this section, focus shifts to social interactions with the perhaps most important counterpart of domesticated animals – the human. Extensive research has produced some exciting new aspects on this relationship, and the chapter provides a broad and up-to-date introduction to this.

# 1

# The Study of Animal Behaviour and Its Applications

P. Jensen

## 1.1 Introduction

Most people have a clear conception of the meaning of the word ‘behaviour’, yet it is strikingly difficult to define it in a precise way. Since ethology is the science of animal behaviour, its causation and function, it is worthwhile to start with some consideration of what type of biological phenomena may be included in the concept. In its simplest form, behaviour may be series of muscle contractions, perhaps performed in clear response to a specific stimulus, such as in the case of a reflex. However, at the other extreme end we find immensely complex activities, such as birds migrating across the world, continuously assessing their direction and position with the help of various cues from stars, landmarks and geomagnetism. It may not be obvious which stimuli actually trigger the onset of this behaviour. Indeed, a bird kept in a cage in a windowless room with constant light will show strong attempts to escape and move towards south at the right timing, without any relevant external cues at all.

We would use the word behaviour for both these extremes and for many other activities in between in complexity. It will include all types of activities that animals engage in, such as locomotion, grooming, reproduction, caring for young, communication, etc. Behaviour may involve one individual reacting to a stimulus or a physiological change, but may also involve two individuals, each responding to the activities of the other. And why stop there? We would also call it behaviour when animals in a herd or an aggregation coordinate their activities or compete for resources with one another. No wonder ethology is such a complex science, when the phenomena we study are so disparate.

But how did it all begin and how has ethology developed into the science it is today? This chapter will provide a brief overview of some landmarks in history and of the various fields into which the science has branched over the last decades. The field that will interest us most in this book is of course the application of ethology to the study of animals utilized by man.

## 1.2 The History of Animal Behaviour Studies

No doubt, knowledge of animal behaviour must have been critical for the survival of early *Homo sapiens*. How could you construct a trap, or kill dangerous prey weighing several times your own weight, unless you have a genuine feeling for animal behaviour? So it should not be any surprise that the earliest ‘documents’ available from humans – cave paintings up to 30,000 years old – are dominated by pictures of animals in various situations. Aristotle published written, systematic observations and ideas about animal behaviour more than 300 years BC (Thorpe, 1979).

One of the first to write about animal behaviour in a modern fashion was the British zoologist John Ray. He published in 1676 a scientific text on the study of ‘instinctive behaviour’ in birds. He was astonished by the fact that birds, removed from their nests as young, would still build species-typical nests when adult. Ray was unable to explain the phenomenon, but noted the fact that very complex behaviour could develop without learning or practice. Almost



**Fig. 1.1.** A sow needs no prior experience to be able to construct an elaborate nest before farrowing. ‘Instinctive behaviour’ such as this fascinated early behavioural researchers.

100 years later, French naturalists had an important influence on the development of the science. For example, Charles Georges Leroy, who was not a formally trained zoologist, published a book on intelligence and adaptation in animals. Leroy heavily criticized those philosophers who spent their time in their chambers, thinking about the world, rather than observing animals in their natural environments. Only then, he argued, would it be possible to fully appreciate the adaptive capacity and flexibility in the behaviour of animals (Thorpe, 1979). [Figure 1.1](#) illustrates the typical nest-building behaviour of a sow.

Another 100 years on, two important scientists deserve to be mentioned. The first is the British biologist Douglas Spalding, who published a series of papers on the relationship between instinct and experience. Spalding was way ahead of his time in experimental approaches. For example, he hatched eggs from hens by using the heat from a steaming kettle, in order to examine the development of the visual and acoustic senses without the influences of a mother hen (Thorpe, 1979). The second important scientist is no one less than Charles Darwin.

Darwin is probably the person who has had the most significant influence on the development of modern ethology – in fact on all modern biology. Most people know him as the father of the theory of evolution, which in itself, of course, is the foundation for any study of animal behaviour. However, he also approached the subject more directly, and his last published work in 1872, *The Expression of the Emotions in Man and Animals*, was probably the first modern work on comparative ethology.

### 1.3 The Schools of the 20th Century

In the beginning of the 20th century, behavioural research grew fast. However, the development in the USA and Europe took different directions. American researchers were influenced by the behaviouristic approach, developed by people such as John B. Watson and later Burrhus Frederic Skinner. Their work was focused primarily on controlled experiments in laboratory environments and their subject species *par préférence* were rats and mice. At the centre of their interest were the mechanisms of learning and acquisition of behaviour through reinforcement or

punishment (Goodenough *et al.*, 1993). The behaviouristic research was concerned with finding general rules and principles of learning, and there was a strong belief that such rules were independent of context. Therefore, the evolutionary histories of the study subjects, or their ecological ways of life, were regarded as irrelevant for the research.

In contrast, in Europe the development of the science was dominated by naturalistic biologists, who spent most of their time observing wild animals in nature. Birds and insects were favourite subjects, and these researchers were mostly interested in instinctive, innate and adaptive behaviour. One of the pioneers was Oskar Heinroth, who first started to use the term ‘ethology’ with the meaning we give it today (Thorpe, 1979). The naturalistic behavioural biologists shared an important approach with the behaviourists. They were not particularly interested in mental processes or emotions that may be associated with behaviour. Such processes were often regarded as unavailable for scientific research, since they were not considered to be observable. Only much later has a scientific interest in mental processes emerged, something that will be dealt with more in later chapters in this book.

In the footsteps of Heinroth, we meet two scientists whose influence over modern ethology cannot be overemphasized: Niko Tinbergen in Holland and Britain, and Konrad Lorenz in Austria. Tinbergen developed a field methodology of high exactness. He designed experiments in which details of the environments of free-living animals were altered and their behaviour could be recorded. He was a true pioneer in experimental ethology (Dawkins *et al.*, 1991). Lorenz, on the other hand, did not go much into nature, but rather bred his experimental animals himself and kept many of them almost as pets. He rarely conducted elaborate experiments and was not prone to quantitative recordings. The strength of Lorenz was on the theoretical level. He formulated many of the fundamental ideas in ethology, and developed the first coherent theory of instinct and innate behaviour (Goodenough *et al.*, 1993).

Lorenz and Tinbergen definitely placed ethology on the solid ground of well-accepted sciences when they, together with the German researcher Karl von Frisch, were awarded the 1973 Nobel Prize in medicine and physiology.

## 1.4 Modern Approaches to Ethology

From the 1960s onwards, ethology developed into the science it is today. This was guided to a large extent by the research programme formulated by Tinbergen, which is still generally accepted as the fundamentals of ethology (Tinbergen, 1963; Dawkins *et al.*, 1991). This programme is frequently referred to as ‘Tinbergen’s four questions’ and the four aspects of behaviour that he used to define the field of ethology were:

1. *What is the causation of the behaviour?* The answer to this question refers to the immediate causes, such as which stimuli elicit or stimulate certain behaviour, or which physiological variables, such as hormones, are important in the causation.
2. *What is the function of the behaviour?* In this case, the answer describes how the behaviour adds to the reproductive success, the fitness, of the animal. It therefore has to do with evolutionary aspects and consequences.
3. *How does the behaviour develop during ontogeny?* Studies on this question aim at describing the way behaviour is modified by individual experiences.
4. *How does the behaviour develop during phylogeny?* This is a clearly evolutionary question, and usually calls for comparative studies of related species.

Whereas early ethology was occupied mainly with causation, ontogeny and phylogeny, the research during the 1960s and onwards became increasingly focused on the functional question.

Researchers have outlined new theories on how behaviour evolves through individual selection at the gene level, and have provided formal mathematical models for how the functional aspects of behaviour could be determined. The impact of this approach on contemporary animal behaviour science has been tremendous.

One aspect that was not covered by Tinbergen's questions is what animals perceive, feel and know in relation to their own behaviour. As mentioned earlier, this aspect of animal behaviour was largely considered to be inaccessible to science. However, other scientists have developed methods and concepts to allow investigation into this area. This has led to a new branch of the science, emerging in the 1970s, known as cognitive ethology (Bekoff, 2000) (the word cognition means subjective, mental processes – or thinking).

## 1.5 Applied Ethology

Even early in the development of ethology, it was apparent that the new insights into the biology of behaviour could be of great value in understanding more of the behaviour of domestic animals. This branch of science saw a dramatic expansion as the debate on animal welfare in so-called factory farming (a concept coined by the influential writer Ruth Harrison) took off in the 1960s. However, applied ethology is not only concerned with animal welfare. Let us look at a few areas of interest.

### Welfare assessment

There is no doubt that the welfare of animals on farms, in zoos and in laboratories dominates the interest of most researchers in the area. The problems may be formulated for example like this: many laying hens in the world are kept in cages made of wire mesh, with very little space available for the animals and almost no substrates for carrying out many of the species-typical behaviour patterns of poultry (see Fig. 1.2). So, which are the most essential behaviour patterns for laying hens? Perhaps it is being able to dust-bathe, or to perch during night, or to perform nest building and lay eggs in a secluded area. All these are typical poultry behaviours, and there may be others as well. How are the animals affected if they cannot behave like this? Can the activities be rated in any order of importance to the animals (Appleby *et al.*, 1993)?

On the other hand, a common alternative to battery cages is a floor system with thousands of hens in one big group, sometimes with quite high stocking rates. In this situation, some unwanted behaviour (which can be present both in cages and in floor systems), such as feather-pecking or cannibalism, might cause great harm to the animals. So, is it better for the hens to be



**Fig. 1.2.** Battery cages (a) and floor housing systems (b) both cause behavioural problems for laying hens. To estimate the relative importance of different behaviours to animals, thereby allowing better decisions regarding choice of housing systems, is one important goal of many researchers in applied ethology. (Photos courtesy of Per Jensen.)

in a situation where they can perform all the activities mentioned above, but where the social system may collapse and abnormal behaviour may spread widely (Hansen, 1994)?

Difficult questions such as these are important aspects of welfare assessment – only rarely do all indices point in the same direction. In this book, Chapter 9 will examine these aspects further and describe some of the methods researchers have developed to try to answer such questions.

## Optimizing production

Farm animals are kept to produce food and other essentials for humans, and farmers need their enterprises to be profitable. It is therefore important that the difference between the value of what the animals produce (e.g. amount of milk or meat) and the cost the farmer incurs for this production (e.g. feed, investments and labour) is sufficiently high.

By taking knowledge of animal behaviour into account, such optimization may be easier to achieve. For example, animals may utilize their feed better if they are fed according to their species-specific feeding rhythm and in a social context that is adapted to the species (Nielsen *et al.*, 1996). Social animals may eat more and digest the food better when all in a group are allowed to feed at the same time.

Social animals that are kept in individual housing systems may be poorer at transforming feed into valuable products. Likewise, husbandry routines applied at a biologically inadequate time may decrease the production rate of the animals. Young piglets that are weaned from their mothers too early and in an abrupt manner show a decreased growth curve, and mixing of piglets after weaning may also have negative results on production (Algers *et al.*, 1990; Pajor *et al.*, 1991).

## Behavioural control

The essence of keeping animals in captivity is to control their behaviour – by preventing them from escaping, to control their breeding and making them adapt to the housing environment. The control is achieved largely by direct human actions, but also by using technical equipment.

A growing interest has been paid to the nature of human–animal interactions. For example, researchers have investigated how animals perceive humans and how they remember experiences with human behaviour. This may help farmers and others to interact more smoothly with their animals (Hemsworth and Barnett, 1987).

An increasing trend in animal farming is to use technical inventions in animal husbandry. For example, group-housed pregnant sows are often fed from an electronic feeding station, which the animals to some extent are required to control themselves. The sows are equipped with transponders that allow them to open the feeding stations and obtain their individual feed rations. However, such systems must be carefully designed to avoid problems. For example, the social hierarchy of a group of sows may lead to some individuals occupying the feed entrance, biting and wounding other animals and thereby destroying the functionality of the system. By means of ethological knowledge, technical equipment can be designed to work better for the animals (Broom *et al.*, 1995).

## Behavioural disorders

Housing systems such as those described earlier, malfunctioning technical equipment or poor human management may all lead to various behavioural disorders. Aggression levels may become excessively high, dramatic behaviour such as cannibalism may develop, and several other types of abnormal behaviour may be seen as well (Lawrence and Rushen, 1993). This is not only the case for farm animals. Many pets develop unwanted and abnormal behaviour, such as owner-directed aggression, uncontrolled urination and defecation in the home, or anxiety-like states.

The characterization and understanding of abnormal behaviour is a central aspect of applied ethology. Sometimes the behaviour can be cured by behavioural therapies, such as enrichment of the home environment or stimulation of other behaviour. At other times, research can provide insights that may help prevent the behaviours from ever developing.

## Behaviour and conservation biology

Animal species are going extinct or becoming threatened at an accelerating rate, largely as a consequence of human activities. This includes hunting, pollution, climate change, habitat loss and introduction of foreign species and diseases. Any countermeasure against this will be greatly helped by a thorough understanding of the behaviour of the species under concern.

There are essentially two main fields of animal conservation biology. In *in-situ* conservation, the focus is on improving the environmental conditions in the natural habitat of the species to the extent that the species will be able to survive where it lives. In *ex-situ* conservation, on the other hand, animals are taken from the wild to be reared and bred in captivity with the goal of eventually reintroducing their progeny when suitable habitats are available.

For *in-situ* programmes, knowledge is required about, for example, normal social structures, foraging strategies and mating behaviour of the species. Unless such things are known, efforts to rescue the environmental conditions may prove futile, since it is not possible to prioritize measures in relation to the needs of the species. Furthermore, behaviour can provide essential indicators for monitoring populations (Berger-Tal *et al.*, 2011). Knowing the normal movement patterns, vigilance and territoriality of a species can provide alarm signals when something in the habitat causes drastic changes in the population.

With respect to *ex-situ* conservation, the above-mentioned topics of ethological knowledge are of course equally important but, on top of that, applied ethology can provide other important insights. For example, the captive environment will always be a compromise between the needs of human caretakers and those of the animals. In addition, not only is behaviour important to consider, but also things such as health and hygiene. Sometimes, the ambition to keep animals fit and free from disease can be in conflict with their behavioural needs. It may be difficult to offer the right food in the species-specific manner, and the possibility to use sufficiently complex cage environments may be in conflict with cleaning needs. Here, applied ethology can offer helpful insights to aid in designing the best captive environments possible.

A fine example of successful contributions from ethology to animal conservation is the case of the North American whooping crane (see Fig. 1.3), which at one point was almost extinct. An extensive *ex-situ* conservation programme utilizing ethologically founded rearing methods was coupled with ambitious *in-situ* field studies after reintroduction to gradually improve the breeding (Kreger *et al.*, 2005). This has led to an impressive recovery of the population.

## 1.6 The Field of Applied Ethology

As should be clear from these accounts, ethology is a science that may offer many different sorts of applications in situations where humans utilize or are responsible for animals for various purposes. Whereas animal welfare assessment clearly dominates in the applications of this science, it is by no means the only way in which knowledge of behaviour can be used.

Applied ethologists are normally concerned with all four of Tinbergen's questions. The causation and ontogeny of behaviour are essential aspects of understanding, for example, how abnormal behaviours develop and how they can be prevented. Phylogeny and function of behaviour are often less emphasized, but many studies have advanced our understanding of domestic animal behaviour greatly by considering how it can have evolved in the ancestors and how it may





**Fig. 1.3.** The whooping crane, a charismatic North American bird species (a), was saved from extinction after intense work with *ex-situ* breeding and ethological fieldwork (b) to get a detailed picture of the natural behaviour of the species. (Photos courtesy of Inma Estevez (a) and Michael Kreger (b).)

have been affected by domestication (Fraser *et al.*, 1995). Experimental studies tend to dominate, but important scientific data have been made available through studies of domestic animals in wild-like conditions (which will become obvious in Chapters 11–19, where accounts are given of the normal behaviour of some important domestic species).

In the optimal situation, applied ethology research concerns all the fields outlined above. They are all interlinked: poor human–animal, or equipment–animal, interaction may cause poor welfare, which in turn leads to behavioural disorders and reduced production, or reduced ability to reproduce in captivity in the case of *ex-situ* conservation. Applied ethology is therefore an essential part of the proper keeping and rearing of animals. And last but not least: as will become obvious in this book, understanding the behaviour of domestic animals is a fascinating aspect of biology in its own right.

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# GLOBAL CLIMATE CHANGE AND COASTAL TOURISM

RECOGNIZING PROBLEMS, MANAGING  
SOLUTIONS AND FUTURE EXPECTATIONS

EDITED BY **ANDREW JONES** AND **MICHAEL PHILLIPS**



2017  
INTERNATIONAL YEAR  
OF SUSTAINABLE TOURISM  
FOR DEVELOPMENT



# 2 A Rapidly Changing Climate in an Era of Increasing Global Carbon Emissions

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## Introduction

Humanity continues to blaze the path towards the increased extraction and burning of fossil fuels without a full understanding of its consequences. With seemingly no end to this finite resource, new drilling and increased extraction opportunities have brought the price of this commodity down. Meanwhile, current assessments on the impact of increased levels of CO<sub>2</sub>, which are primarily generated from the burning of such fuels, point towards the consequential effects of extreme natural events in light of heatwaves and droughts, heavy rainfall, floods and sea level rise on communities (IPCC, 2014). Climatologists highlight the urgent need to cut down drastically CO<sub>2</sub> emissions in view of the longevity of airborne carbon present in the atmosphere (Archer, 2005) and have subsequently placed a red flag on the resulting persistence of the induced warming (Solomon *et al.*, 2010) which can entrench inevitable and highly undesirable consequences.

## Vigilance of the Climate System

The climate system is defined as an interactive and multi-component system consisting of

the atmosphere, the hydrosphere, the cryosphere, the land surface and the biosphere, all of which are influenced by various external forcing factors, such as the sun. The direct effect of human activities on the dynamics of the climate system is termed as internal forcing (IPCC, 2016).

The vigilant monitoring of the climate system is continuously garnering new empirical evidence of its rapidly changing nature. The future prediction of a positive trend in global warming from increasing atmospheric levels of greenhouse gases (GHGs)<sup>1</sup> is now robust (IPCC AR5, 2014). This change is quickly moving outside the boundaries of human experience, with the occurrence of unpredictable, geographically distant and disastrous events, which are already stressing societies around the world (NOAA, 2016). This does not exclude the fact that cyclic fluctuations of our climate during the past million years did not disrupt salient land and ocean processes. There is now ample scientific evidence to prove that the climate with time widely fluctuated between Ice Ages and warm interim periods, as a result of which there have already been major biological extinctions.

So why is so much concern given to our climate system in view of its perennially

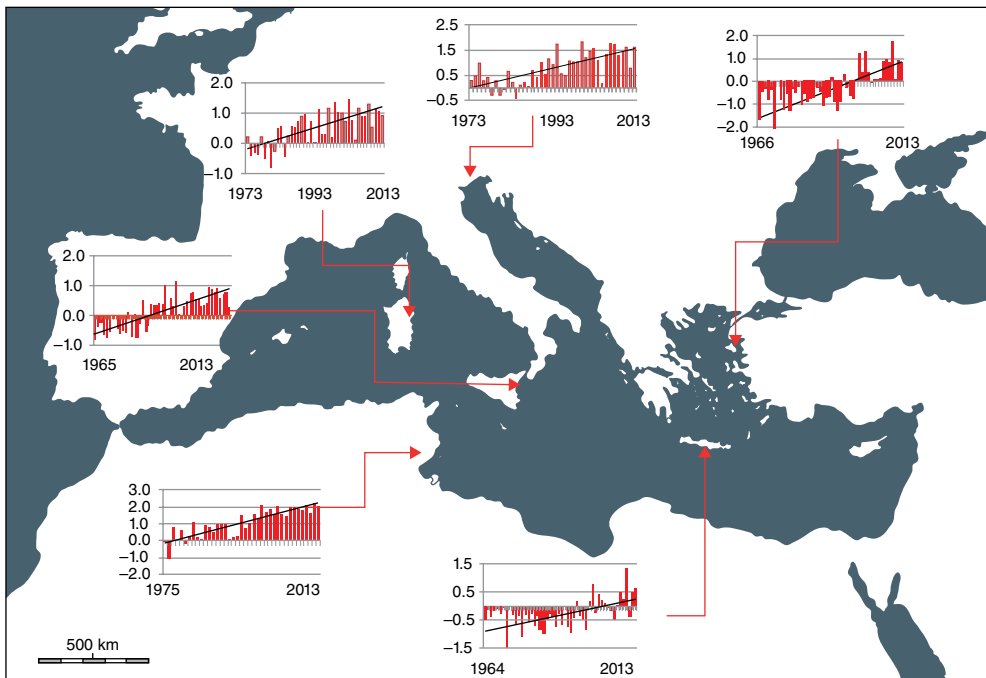
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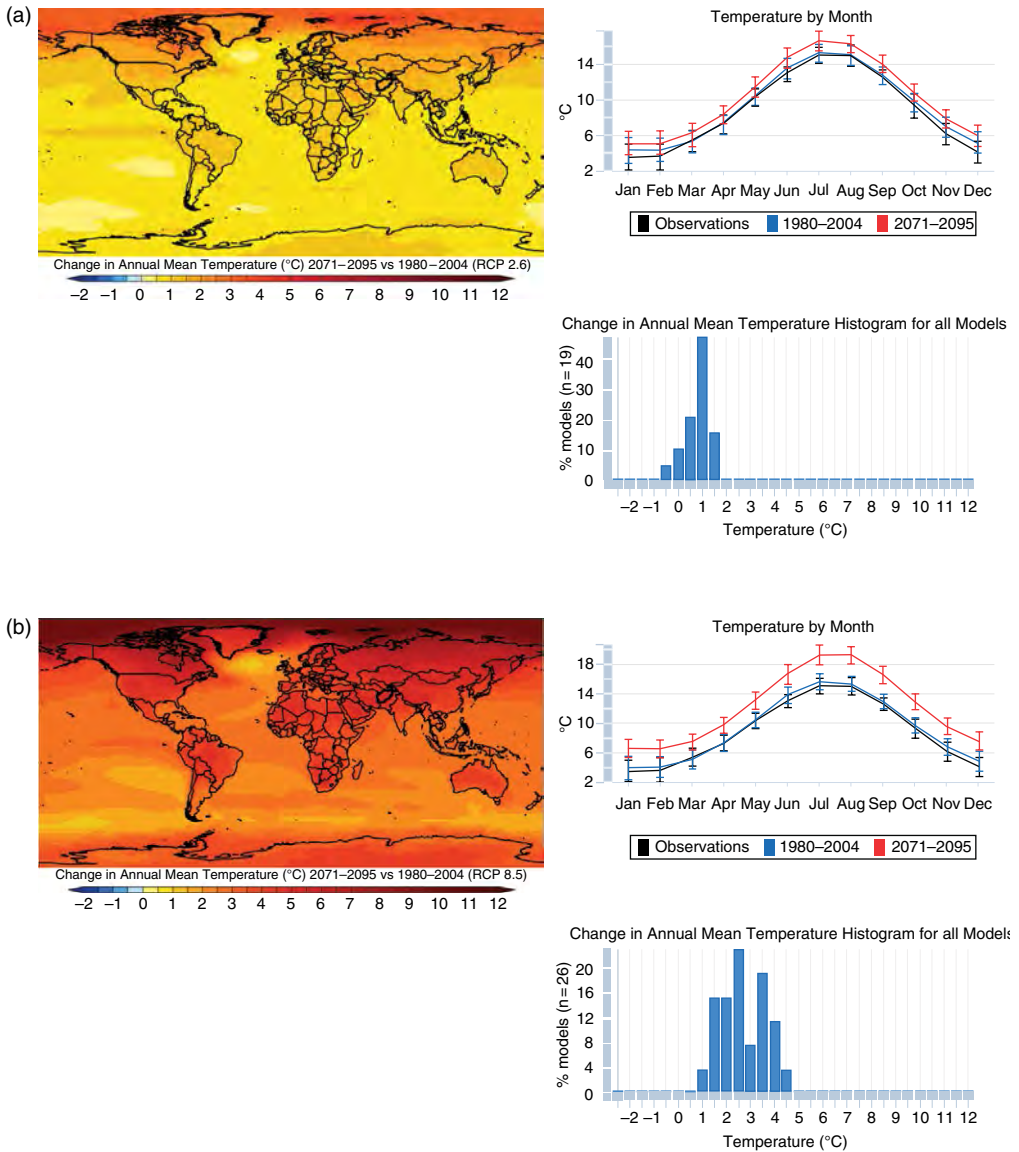
changing nature? Put succinctly, the difference between today's global temperature and the last Ice Age<sup>2</sup> lies only in an increase of 5°C. As a result the answer to this legitimate question is provocatively straightforward; one need only focus on this temperature range and compare it with the present-day rate of temperature increase. The similarity in the variation between the current post-glacial temperature (i.e. pre-industrial global air temperature) and the predicted increase in global temperature up till 2100<sup>3</sup> under moderately high anthropogenic forcing conditions is striking (IPCC, 2013). One may instantly realize that the rate of increase in temperature will occur in just over 100 years as opposed to 7000 years. The airborne CO<sub>2</sub> concentration that has already accumulated in the atmosphere will continue changing the Earth's radiation budget in centuries to come, let alone its expected increased levels.<sup>4</sup>

So far the *global* temperature anomaly of 0.87°C since 1880 is considered to be quite significant. In certain parts of the world, such as the central Mediterranean (Fig. 2.1), this rate of increase has been shown to be higher during recent years (Galdies, 2012).

The latest 'long-term' simulations of future climate were produced by the Coupled Model Inter-comparison Project Phase 5 (CMIP5).<sup>5</sup> Simulations are made of both the 20th century (based on past, natural and anthropogenic forcing) and of the 21st-century climate (based on four assessments called Representative Concentration Pathways – RCPs; van Vuuren *et al.*, 2011). CMIP5 simulations of the global annual mean temperature show that it is expected to increase over time (Fig. 2.2), especially over particular geographical regions. For example, under the RCP 2.6 scenario the annual mean temperature values produced by the HadGEM2-ES



**Fig. 2.1.** Temperature anomalies at the Mediterranean level based on synoptic meteorological observations by WMO climate stations (1960s–2014). Inset graphs show temperature anomalies at various WMO Climate Centres in the region and thus climatic variability of the region (Galdies, 2015). WMO recommends 30 years as a standard period for the analysis of climate anomalies (Folland *et al.*, 1990), and the climate period between 1961 and 1990 at the individual locations was used as a typical baseline to calculate site-specific temperature anomalies, in conformity with the IPCC and other official Climate Centres.



**Fig. 2.2.** (a) projected change (°C) of the mean temperature surface air temperature at the end of this century (2075–2095) relative to the recent past (1986–2005) for the lower RCP 2.6 (top left) and (b) for the higher RCP 8.5 scenarios (bottom left). Individual figures were generated by the author and adapted using USGS CMIP5 Global Climate Change Viewer. Both projections shown have been produced by UK's HadGEM2-ES climate model. The sets of graphs on the right show the resultant mean monthly temperature produced from all CMIP5 models for the UK (Alder and Hostetler, 2013; Alder *et al.*, 2013).

model are projected to increase especially over the northern hemisphere, unlike that simulated under RCP 8.5, which shows an overall increase of temperature worldwide. Taking the UK as an example, the projected

mean CMIP5 model temperature increase is expected to be between 2°C and 8°C (Fig. 2.2) depending on the RCP used by the model.

The crux of the matter lies in the speed at which the current climate is changing in

that it is greater than any previous changes detected in what are known as *proxy* indicators of the ancient climate (such as ice- and sediment-cores). Suffice to say that at the time of writing the current global concentration of CO<sub>2</sub> to 405.75 ppm<sup>6</sup> was last seen around 15 million years ago<sup>7</sup> (Tripathi *et al.*, 2009). This rapid perturbation to the entire climate system is making it more difficult for both humankind and nature itself to adapt quickly to altered states of its various components. While climate sceptics invoke the role of natural forcing behind such a rapid change, an overwhelming majority of scientists rule out any natural changes in external forcing by the sun, volcanic activity, or variations such as the El Niño-Southern Oscillation (ENSO; EPA, 2016) as being the main culprit of the present change in the climate.

The long-term, irreversible shift of the climate system as a response to continued carbon emissions is now well documented in the scientific literature (IPCC, 2013). A total of five IPCC assessments since 1990 have mainly assessed the projections of climate change and their impact for the 21st century. The group of experts responsible for these two tasks continues to uphold its views on matters relating to the time lag invoked by an increasing carbonized atmosphere, in other words, even if carbon emissions are kept constant or reduced by 2100, atmospheric CO<sub>2</sub> concentrations and surface temperatures would remain high, and that sea level would continue to increase for thousands of years to come. This situation will remain so unless an efficient and large-scale carbon sequestration and storage mechanism is used to immediately capture all of the surplus airborne carbon as well as that produced at source. Carbon sequestration occurs very slowly by a variety of natural processes, which stands in stark contrast with the hundreds of millions of years in order to produce the fossil fuels reserves we have at our disposal today (National Research Council, 2015).

Ironically, the advance in climatology is sometimes impacted by perplexing discoveries that challenge our current understanding of the magnitude of anthropogenic forcing on our climate system. The scientific process is obliged to unravel such conflicting results

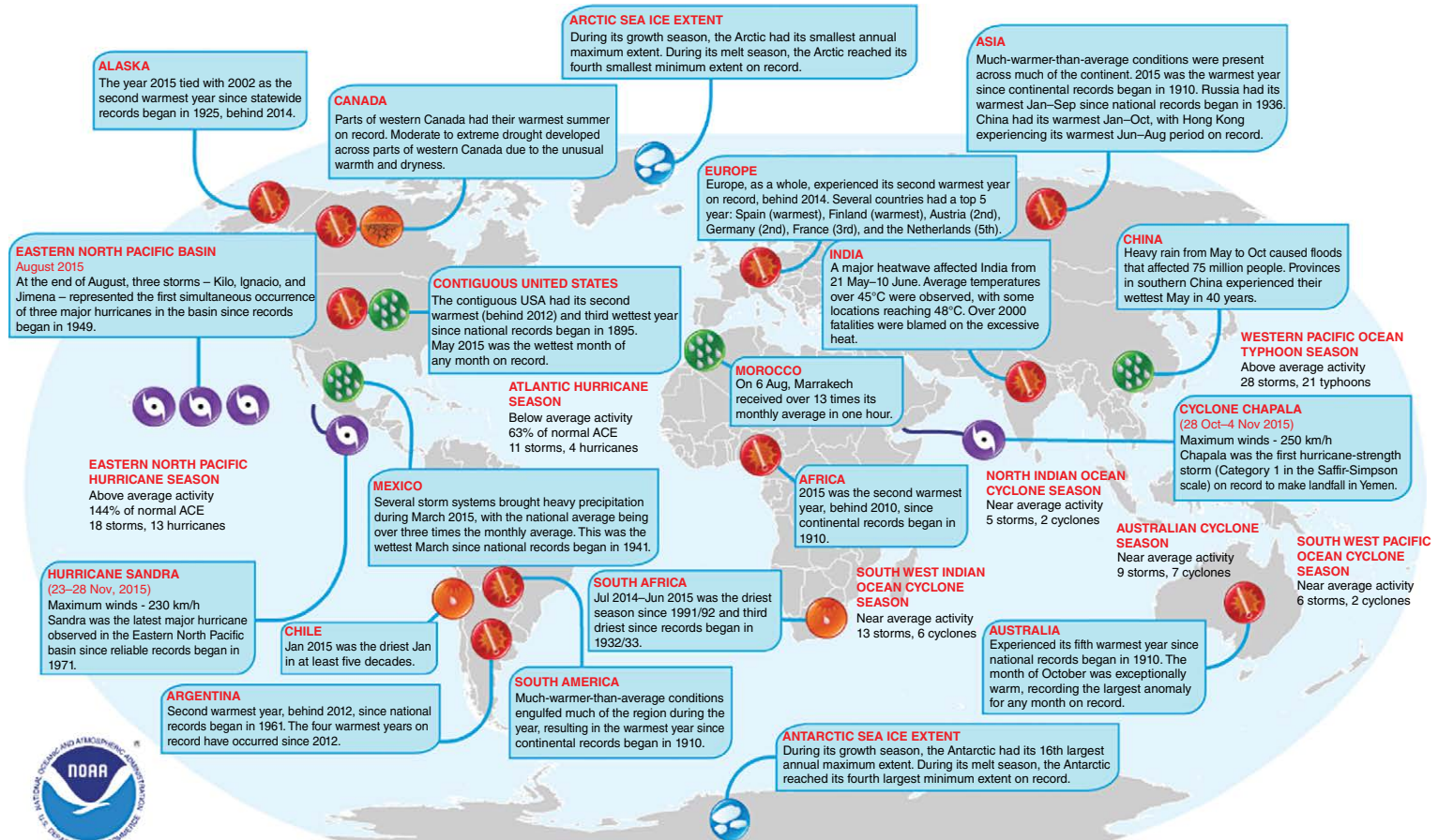
and review them in the light of further experimentation and understanding. Xie (2016) took up this important task by attempting to explain how the observed recent slowdown in global warming (better known as the ‘climate hiatus’) came about during periods of increased carbon emissions. This occurred at a time when the rate of global mean temperature decreased by a factor of two during the period 1998–2012 when compared to the previous 15 years. This has of course become embroiled in scientific and political debate (Fyfe *et al.*, 2016).

Xie (2016) documented the required theoretical and practical studies and observations made on Earth’s energy balance of the ocean in order to explain this discovery. This thorough analysis has placed the tropical Pacific decadal variability<sup>8</sup> in an important position as a modulator of the climate system and as being the main cause of this climate hiatus; however, its mechanisms still need to be elucidated. For many researchers this unexpected discovery was seen as an opportunity for further research, highlighting the need to continue understanding the climate system as a precondition for its accurate prediction.

### Climate Change Impacts and their Implications on Global Security

Anthropogenic climate change has been linked to an increase in the frequency and intensity of a range of disruptive environmental events (IPCC, 2014). Such events are already discernible and causing stress to communities (Fig. 2.3) in the form of damage to local and globally integrated systems that support human well-being, such as public health (Mellor *et al.*, 2016), ecosystem health and services, food supply (Tripathia *et al.*, 2016) and infrastructure. NASA’s Global Climate Change Portal (2016) cites the lengthening of growing season, changes in precipitation patterns, increased incidences of droughts and heatwaves, intensification of hurricanes, sea level rise, and de-icing of the Arctic Ocean as distinctive current and future impacts. Another equally significant impact

## Selected Significant Climate Anomalies and Events in 2015



Please Note: Material provided in this map was compiled from NOAA's NCEI State of the Climate Reports and the WMO Provisional Status of the Climate in 2015. For more information please visit: <http://www.ncdc.noaa.gov/sotc>

Fig. 2.3. Selected significant climate anomalies and significant events during 2015 (NOAA Global Climate Change portal: Global Analysis – Annual 2015).



is mass extinction, brought about by the inability of the biosphere to adapt so quickly to new climatic conditions. On land, shifts in the timing of the seasons and life-cycle events such as blooming, breeding and hatching are causing mismatches of biological interactions that disrupt patterns of feeding, pollination and other key aspects of food webs (Tripathia *et al.*, 2016). In the oceans, ocean stratification is increasing resulting in less water-mixing between the upper warmer and cooler, deeper waters (Hansen *et al.*, 2016) and linked to an inability for marine life such as phytoplankton found near the surface to access nutrients from below. Phytoplankton constitutes the very foundation of the ocean food web. Readers should refer to additional sources of information for additional impacts such as ocean acidification, coral bleaching and increased incidence of vector-borne diseases.

Adaptive governance requires adequate information on the nature of these impacts in order to respond effectively to related risks. Significant improvements have been made in quantifying future temperature trends on the basis of the current and future emission scenarios of GHGs, and are now helping policy and decision makers to act accordingly. From this adaptation standpoint, the main requirement needed is the evaluation of expected risks by taking into account a range of possible future climate conditions and their associated impacts, and whether the cost of limiting the harm is acceptable. However, due to the current limitations in technology and knowledge of the climate system it is still not possible to predict the precise timing, magnitude and location of these events from decades in advance; there is instead considerable improvement in the equally useful knowledge concerning their nature, risk and social vulnerability.

A timely publication by National Research Council (2013) describes how the initial impact of a warming climate takes the form of disconnected clusters of extreme events. However, with time these sporadic extreme impacts will start taking their toll on integrated socio-economic and environmental systems, which can potentially trigger an internal cascade reaction of negative events

with yet unknown consequences if their exposure and susceptibility are sufficiently high and repetitive, and our response is inadequate. Under such situations, national security might be better off if it applies a scenario-based approach of altered integrated systems rather than focus on *isolated, individual extreme* events, which perhaps are more predictable. In this context, experts argue that it would now be more appropriate to consider security risks that are able to disrupt, even at low but chronic levels, the internal linkages of global systems rather than simply prioritize risk on the basis of the magnitude of these extreme events. Therefore understanding how integrated systems are internally connected and how vulnerable they are to impacts of climate change is without a shadow of doubt the most important aspect of climate change from a national security point of view. Incidentally, this approach has so far received very little scientific attention (National Research Council, 2013).

### Current Quick Fixes

Despite warnings of impending climatic extreme events, fossil fuels remain the world's primary energy source. Economic and technological progress continue to determine significantly CO<sub>2</sub> emissions (Kais and Sami, 2016). Solace in minimizing the effects of climate change is being sought in the slow but continuous application of mitigation measures and by the use of advanced technologies that are able to harness energy from renewable sources. This strategy started since UNFCCC came into force in 1994 and is periodically revisited at the Conferences of the Parties (COP) to the UNFCCC by means of lengthy negotiations aimed at keeping to the pre-determined emissions goals till 2100. Such targets are indeed very important and are considered as tangible steps towards shifting towards clean technologies, energy efficient processes and a low carbon economy.

However, this strategy fails to recognize the nature of the threat posed, the recognition of which many top climatologists ascribe as

being too little, too late. Clark *et al.* (2016) argue that assigning emission targets and introducing new technologies is short-sighted and is unlikely to have a major impact on emissions, especially if the general ‘political’ perspective of anthropogenic climate change continues to limit itself till 2100.

Climatologists view this perspective as a political and strategic gridlock, which in turn myopically identifies the climatic problem as being only relevant to the next 100 years, and that the expected negative impacts of a changing climate can be reversed if cuts (which some consider to be not overly drastic) are made to current rates of emissions. Consequently, the current proposals and agreements to drastically curb emissions remain limited in scope and effectiveness simply because they are short-term in nature and do not take into account the lag of the climate system to respond to the *current* levels of GHGs in the atmosphere (Clark *et al.*, 2016). Even if we were to entertain the idea of keeping to the current rates of GHG emissions,<sup>9</sup> the climate would still continue to warm up well beyond the 0.87°C already observed and capable of drastically changing its dynamics for the next 10,000 years.

With the help of historical data going back hundreds of thousands of years and the latest climate models, scientists are now able to obtain highly probable long-term scenarios even if their precise timing remains uncertain. Their findings point towards the need for humankind to act fast and bring about the necessary change by 2040.

An interesting study that is hot off the press is a study made by Hansen and 18 other co-authors published this year in the journal *Atmospheric Chemistry and Physics*. They used climate simulation models, palaeo-climatic data from the past 120,000 years and modern observations to study the effect of growing ice melt from Antarctica and Greenland. Continued high GHG emissions until 2100 are predicted to yield an imbalance in the ocean heat budget and to the global thermohaline circulation in such a manner as would result in increasingly powerful storms and an exponential increase in sea level rise. Similar conditions have prevailed some 120,000 years ago, during which

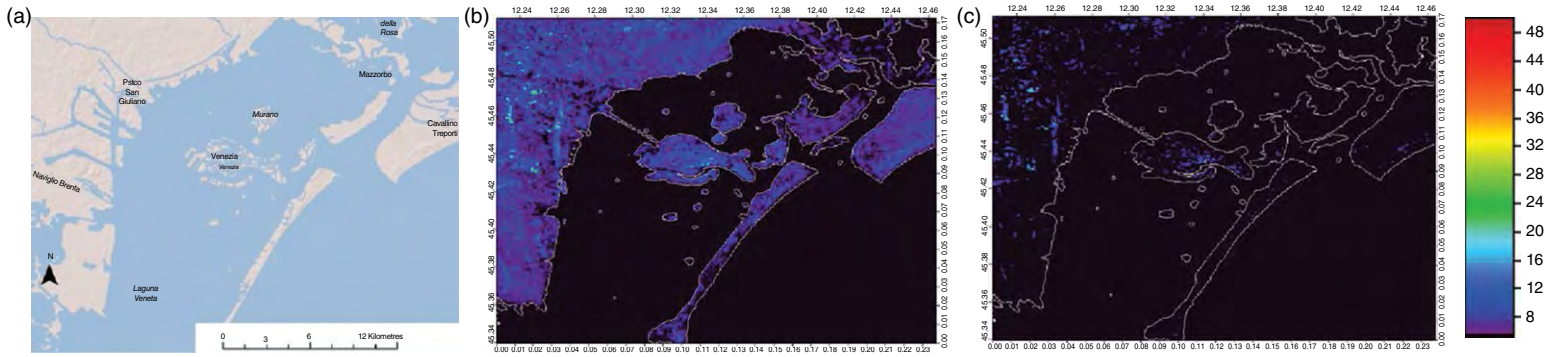
huge swaths of polar ice melted down and resulted in a sea level rise of from 6 to 9 m (Hansen *et al.*, 2016). Nothing new so far; sea level rise is a sure thing that is already ongoing. However, this study presents a sea level rise scenario that is diametrically opposed to what we have been hearing so far – one which will only require 50 years to unfold and not centuries. Results also show that this expected melting will be exponential. The resultant costs incurred by coastal communities and megacities are yet unknown.

Realistic visualizations of the impact of sea level rise can be derived from inundation maps modelled for important, low-lying coastal areas. Venice lagoon, for example, represents a focal area of immense art and cultural value because of its unique location, landscape, as well as its cultural history. One can process satellite-derived topographic data (ASTER GDEM) to simulate the projected impact of sea level rise within the lagoon (Fig. 2.4). With a total sea level rise of 5 m (as modelled under RCP 8.5 conditions), much of the current coastline within the lagoon area will already be under water.

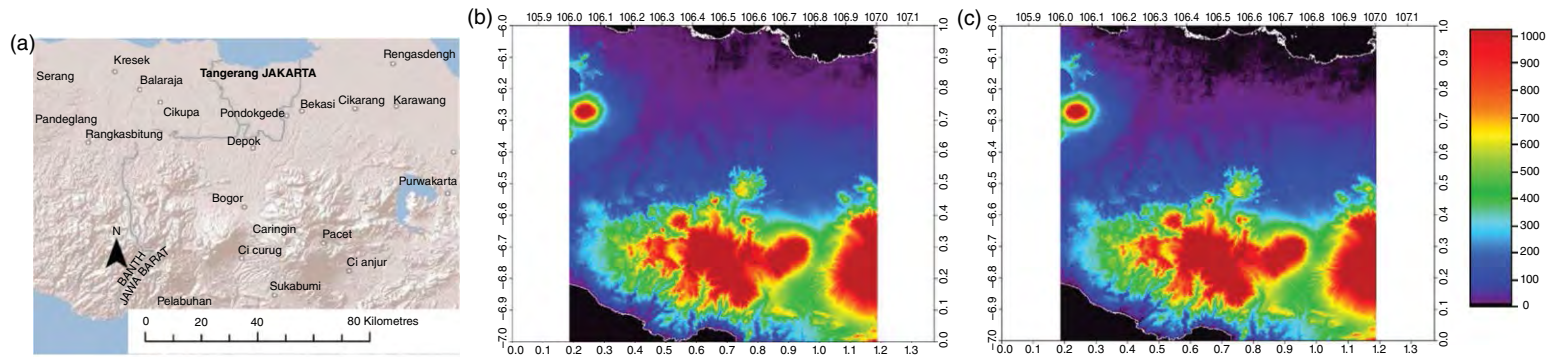
Huge tracts of low-lying countries (some of which below sea level) such as Belgium may not survive climate change. On 24 March 2016, Nicholas Kristof from the *New York Times*<sup>10</sup> argued to having a political system that is as sensitive to such risks as it is to homeland security. Clark *et al.* (2016) illustrated how 50–75% of the people living in Indonesia and residing in low-lying areas will eventually be submerged (Fig. 2.5).

### Short- Versus Long-term Actions

It is important that policy makers now place what we have learnt so far about the climate system, i.e. since the last Ice Age till the next 10,000 years, into an appropriate climate action. The sole consideration of a 250-year period since pre-industrialization is inappropriate since it is unable to account for all possible perturbations of the climate system as a response to increased rates of CO<sub>2</sub>. Climatologists instead are forcing policy makers to



**Fig. 2.4.** Inundation maps of part of the Venetian Lagoon at varying sea level rise. Legend shows height above sea level in metres. Topographic data are based on ASTER GDEM data (ASTER GDEM is a product of METI and NASA). (a) Current coastline of the Venetian Lagoon, Italy; (b) 5 m above current sea level; and (c) 10 m above current sea level.



**Fig. 2.5.** Inundation maps of part of Jakarta area (Indonesia) at varying sea level rise. Legend shows height above sea level in metres. Topographic data are based on ASTER GDEM data (ASTER GDEM is a product of METI and NASA). (a) Current coastline of Jakarta area, Indonesia; (b) 5 m above current sea level; and (c) 10 m above current sea level.

opt for complete de-carbonization of the world's energy systems as soon as possible, which is different from IPCC's efforts to reduce global GHG emissions by at least 50% until 2050 (Clark *et al.*, 2016; Zhou and Wang, 2016). Irrespective of the time frame, a 'carbon quota' on future cumulative CO<sub>2</sub> emissions should already be in place in order to keep the global temperature to 2°C from pre-industrialized temperatures, which would correspond to a global mitigation rate of over 5% per year with a 50% probability of success (Raupach *et al.*, 2014). In quantitative terms, this amounts to a remaining emission quota of 1500 GtCO<sub>2</sub> starting from 2015, or of 1300 GtCO<sub>2</sub> as of 2020 (Friedlingstein *et al.*, 2014). This quota implies that near future cumulative CO<sub>2</sub> emissions consistent with a given warming limit will be a common and finite resource that must be somehow shared among countries on the basis of equity, and institutionally guided by an international policy, economics and financing.

There are several key opportunities to achieve the expected high mitigation rate. These range from land-based bio-sequestration or bioenergy with carbon capture and storage to the use of low- and zero-carbon economy. Interim opportunities that limit demand for goods and services that require energy as well as improving energy efficiency should be sought, such as: (i) changes in abatement technologies, fuel quality and fuel switching; (ii) changes in the structure and efficiency of the energy systems; and (iii) changes of the total economic activity (Andreoni and Galmarini, 2016). Governments have a pivotal role to play in influencing key stakeholders through effective policies and incentives addressing both private sector investments and consumer behaviour, backed by complementary policies.

The 2015 Paris COP 21 merits a special mention. For the first time, producers of 20% of the world's oil and gas (including BP, Shell, Saudi Aramco and Total, among others) expressed their willingness to assist in the 2°C temperature limit by committing themselves to reduce the GHG intensity of the global energy mix. This will be done

through improved efficiency of their own production by giving preference to natural gas over coal, by investing in carbon capture and storage as well as through the harvesting of renewable energy.

A paradigm shift is needed in order to implement the COP 21 agreement's ambition (Obergassel *et al.*, 2016) and its fate is linked to the decoupling of economic growth and the use of fossil fuels as early as possible. However, critics of the Paris Agreement expressed that the financial part of this agreement is weak and does not go beyond what has already been agreed at the Copenhagen Summit in 2009 to mobilize an annual US\$100 billion of financial flows by 2020 and beyond. Its effectiveness depends on whether the momentum gained during the December 2015 Conference can be translated into a major political force. This depends entirely on the willingness and rapidity of nations to fulfil their declared pledges.

## Conclusion

So far the journey from the 1997 Kyoto Protocol to the Paris 2015 Agreement has been problematic and more work remains on the implementation of binding agreements. Decoupling economic growth from continued carbon emissions is an ambitious objective at all three national, international and supra-national levels (OECD, 2013). This chapter discussed the reason why there should be a drastic reduction of GHG emissions. Environmental economists support a system of 'negative emissions' up till 2050 in order to limit warming to 2°C over pre-industrial levels by the end of the century (Zhou and Wang, 2016). Huge capital investment in large-scale technologies such as carbon sequestration and storage, assisted by massive harnessing of renewable energy, could make this possible. At the same time, another important fact has to be considered in all major decisions: an ever-growing global population that needs food, energy, access to basic necessities, and an environment that promotes economic growth and social justice.

## Notes

<sup>1</sup> GHGs are referred to as positive forcing agents because of their ability to shift the planet's energy balance towards the higher side of internal energy. The forcing power of every GHG can be calculated on the basis of their atmospheric levels over time and on their energy transfer through the atmosphere.

<sup>2</sup> The level of atmospheric CO<sub>2</sub> was higher in the distant past, with higher global temperatures and sea level. The CO<sub>2</sub> level in the atmosphere reached today's levels some 3–5 million years ago, a period when global average temperature is estimated to have been about 2–3.5°C higher than in the pre-industrial period. The level of CO<sub>2</sub> may have even reached 1000 ppm around 50 million years ago, linked to a global average temperature of around 10°C higher than the current one. Under those conditions, the sea level was at least 60 m higher than it is today (Clark *et al.*, 2016).

<sup>3</sup> Currently the temperature anomaly for the period 1880–2015 stands at 0.87°C when compared to the 1951–1980 baseline reference. [http://climate.nasa.gov/system/internal\\_resources/details/original/647\\_Global\\_Temperature\\_Data\\_File.txt](http://climate.nasa.gov/system/internal_resources/details/original/647_Global_Temperature_Data_File.txt) (accessed 17 April 2016).

<sup>4</sup> The pathway with the highest GHG emission (i.e. RCP 8.5) represents 'business as usual' – strong economic development for the rest of this century, driven primarily by dependence on fossil fuels, where the concentration of airborne CO<sub>2</sub> exceeds 1000 ppm CO<sub>2</sub> eq. (Riahi *et al.*, 2011).

<sup>5</sup> CMIP5 is an international climate model inter-comparison exercise involving more than 20 modelling groups and over 40 global models. It was set up to provide a basis for coordinated climate change experiments for IPCC's assessment reports through the World Climate Research Programme (WRCP). Its objective is to provide projections of future climate change on a time scale up until 2100 and beyond, and in doing so, understand some of the factors responsible for differences in model projects. <http://regclim.coas.oregonstate.edu/visualization/gccv/cmip5-global-climate-change-viewer/index.html> (accessed 17 April 2016).

<sup>6</sup> [www.esrl.noaa.gov/gmd/ccgg/trends/global.html](http://www.esrl.noaa.gov/gmd/ccgg/trends/global.html) (accessed 17 April 2016).

<sup>7</sup> Here it is important to keep in mind the uncertainties embedded in the techniques used to understand our ancient climate. A high CO<sub>2</sub> level could well have been one of the other key factors in controlling the palaeo-climate.

<sup>8</sup> The Pacific Decadal Oscillation (PDO) is a pattern of Pacific climate variability similar to the El Niño–Southern Oscillation (ENSO) in character, but with a duration of 20 to 30 years, in contrast to ENSO's phase of around 6 to 18 months. The PDO consists of a warm and cool phase, which alters upper level atmospheric winds. It has been found that shifts in the PDO phase can have significant implications for global climate and weather. Experts also believe that the PDO can intensify or diminish the impacts of ENSO (Xie, 2016).

<sup>9</sup> An estimated 374 billion t of carbon has been released to the atmosphere since 1751. Half of the CO<sub>2</sub> emitted by fossil fuels has occurred since the mid-1980s. The 2011 global fossil-fuel carbon emission was 9449 million t of carbon. This represented so far a record high and was 3.4% higher than the global 2010 emissions. These are the latest figures released in the public domain (Boden *et al.*, 2015).

<sup>10</sup> [www.nytimes.com/2016/03/24/opinion/terrorists-bathtubs-and-snakes.html?ref=opinion](http://www.nytimes.com/2016/03/24/opinion/terrorists-bathtubs-and-snakes.html?ref=opinion) (accessed 17 April 2016).

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