

## Bird-mediated selection on fruit display traits in *Celtis ehrenbergiana* (Cannabaceae)

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### ABSTRACT

**Background:** In mutualistic interactions of fleshy-fruited plants and seed-dispersing birds, dispersers act as natural selection agents on fruit display traits. Bird-mediated phenotypic selection on maternal and seed level plant traits has been postulated to be uncoupled. However, this key step in the understanding of the co-evolutionary processes has seldom been explored.

**Goals:** To study the pattern and strength of phenotypic selection exerted by birds on two different plant life stages: maternal (mean and within-plant variation of fruit traits) and offspring (individual seed size) levels.

**Organism:** The one-seeded fleshy-fruited tree, *Celtis ehrenbergiana* (Klotzsch) Liebm.

**Field site:** Natural forests of the Biosphere Reserve ‘Parque Costero del Sur’, near the shore of the Río de La Plata, Buenos Aires, Argentina.

**Methods:** We sampled 24 randomly selected trees. For each focal tree, we recorded bird visits and behaviour, and fruit-related traits. Dispersed seeds were collected at the same location.

**Results:** At the maternal level, birds exerted positive directional selection on mean sugar concentration. Sub-individual variation in fruit traits was not observed to be a target of bird-mediated selection. At the individual seed level, birds exerted positive directional selection on seed size.

**Conclusions:** While birds exert selection pressures on reward-related traits, plants obtain an advantage through a larger seed size.

*Keywords:* co-evolution, maternal and offspring levels, mutualism, natural selection, phenotypic selection, plant–animal interactions, seed dispersal, selection gradients.

### INTRODUCTION

In plant–animal interactions, seed dispersers play a key role in the ecology and evolution of plant populations. On the one hand, patterns of animal behaviour strongly affect post fruit display plant life stages (Schupp, 1995; Jordano, 2000; Schupp *et al.*, 2002; Russo *et al.*, 2006); on the

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other hand, fruit display traits, such as fruit size, seed size, and pulp-to-seed ratio, affect animal foraging behaviour (Jordano, 2000; Izhaki, 2002). Therefore, frugivores are expected to exert selection pressures on plant traits related to seed dispersal (Jordano, 1995). Empirical evidence suggests that fruit-eating birds exert both negative and positive directional selection on fruit display traits (Howe, 1981; Jordano, 1982, 1987; Wheelwright, 1985, 1993; Hedge *et al.*, 1991; Avery *et al.*, 1993; Alcántara *et al.*, 1997; Hampe, 2001; Izhaki, 2002; Galetti *et al.*, 2013). Despite this evidence, the complex nature of the selective pressures and phenotypic evolutionary pathways of fruit traits has seldom been explored (Jordano, 1995; Martínez *et al.*, 2007; Sobral *et al.*, 2010).

Part of this complexity arises from the relationships among fruit traits a plant displays. The fruit crop size hypothesis states that plants offering a high relative number of fruits should attract more frugivores, increasing fruit removal and seed dispersal (Snow, 1971; Howe and Estabrook, 1977; Izhaki, 2002). However, fruits are deemed to be expensive, and the production of more fruits could compromise their quality (Smith and Fretwell, 1974; Mesina and Fox, 2001; Marshall *et al.*, 2010). The conflict of interest between the production of more fruits and the production of fruits of better quality may promote stabilizing selection on fruit crop and individual-level fruit traits (Agrawal *et al.*, 2010).

The strength of selection on fruit crop will also depend on how birds select among fruit display traits. Sallabanks (1993) proposed that frugivores select fruit traits in a hierarchical fashion. Avian frugivores may first use extrinsic plant traits (habitat type, neighbourhood, availability of fruiting plant species) when selecting among fruit crops, and then use intrinsic plant traits [plant size, fruit set, fruit crop, fruit size (Sallabanks, 1993; Jordano and Schupp, 2000)]. By using different criteria at each level of decision, birds may exert different selection pressures on fruit crop and individual-level fruit traits (Jordano, 1995). Thus, selection pressures on maternal traits (crop size, mean fruit and seed size, mean sugar concentration) and offspring traits [individual fruit and seed size, individual sugar concentration (Mojonnier, 1998)] may be uncoupled (Jordano, 1995; Martínez *et al.*, 2007).

The hierarchical nature of selection exerted by birds also suggests that plant–frugivore interactions depend on within-plant variation of phenotypic traits [i.e. sub-individual variation (Herrera, 2009)]. Studies of frugivore-mediated selection on fruit display have generally focused on selection pressures on trait means. Such studies have mostly neglected the adaptive role of fruit trait combinations and sub-individual variation among multiple repeated structures offered by plants (Herrera, 2009). Although Herrera reported selection against fruit width variation in *Phillyrea latifolia* (Herrera, 2009), the mechanisms accounting for bird-mediated selection on sub-individual fruit trait variation deserve more attention. To our knowledge, there have only been four quantitative studies of selection on sub-individual variation and uncoupling of selection pressures (Martínez *et al.*, 2007; Sobral *et al.*, 2010, 2013a, 2013b). Sobral *et al.* (2010) reported a negative correlation between selection on fruit and seed size and selection against fruit diameter variation in *Crataegus monogyna*, suggesting that birds maximize the amount of pulp ingested at the same time as minimizing seed size. These authors also highlight different kinds of phenotypic selection on maternal and offspring fruit display traits, supporting the premise of uncoupled selection pressures related to foraging decision levels. Furthermore, in a recent study accounting for between-population variation in *Crataegus monogyna*, Sobral *et al.* (2013a) revealed a significant relationship between bird-mediated selection and fruit size variation, noting how important it is to consider the sub-individual level as a target of selection.

Current understanding of bird-mediated selection on fruit traits reveals the need to examine the relationships between the type, direction, and strength of selection pressures

according to plant trait type and life stage. Accordingly, we wished to evaluate the role of birds as selection agents on fruit display traits in a population of the one-seeded tree *Celtis ehrenbergiana* (Cannabaceae). Specifically, we addressed the following questions: (1) Does larger fruit crop size have a detrimental effect on fruit quality? (2) What is the strength of sub-individual variation in fruit traits? (3) What is the pattern and strength of the selective pressures exerted by birds on both maternal (mean and sub-individual variation) and offspring traits? (4) Are the selective pressures acting on maternal and offspring traits coupled?

## METHODS

### Study area

The study was carried out at the private El Destino reserve (35°08'S, 57°25'W) and the San Isidro ranch (35°09'S, 57°23'W), both located in the Biosphere Reserve 'Parque Costero del Sur', near the shore of the Río de La Plata, Buenos Aires province, Argentina. The area is composed mostly of grasslands but with nearly 10% forest cover (Cueto and López de Casenave, 2000a). In eastern Buenos Aires province, *Celtis ehrenbergiana* forms xeromorphic forest locally called 'talares'. These 'talares' grow on calcareous soil deposits, parallel to the shore of the Río de La Plata (Goya *et al.*, 1992). In phytogeographic terms, this area corresponds to the austral limit of 'Provincia del Espinal' (Parodi, 1940; Cabrera, 1971). In these forests, the dominant tree species are *Celtis ehrenbergiana*, *Scutia buxifolia*, and *Schinus longifolia* (Cueto and López de Casenave, 2000a; Torres Robles and Arturi, 2009). The dominant shrubs are *Pavonia sepium* and *Sida rhombifolia*, and the most common herbs *Oplismenopsis najada*, *Parietaria lusitanica*, and *Euphorbia chilensis* (Cagnoni *et al.*, 1996). The climate is wet temperate, with mean minimum and maximum temperatures of 5.9°C and 27.5°C, respectively (Cueto and López de Casenave, 2000b). Annual mean rainfall is about 885 mm, mostly wet in January and February, but without a noticeable dry season (Cueto and López de Casenave, 2000a).

### Study system

*Celtis ehrenbergiana* (Klotszch) Liebm. (Cannabaceae) is a thorny andromonoecious tree, found in dry forests and scrubs from the southern United States to central Argentina (Berg and Dahlberg, 2001). It produces little actinomorphic, greenish or yellowish, hermaphroditic or male flowers (Hunziker and Dottori, 1976) and is characterized as an insect- and wind-pollinated self-compatible species (Basilio and Torretta, 2006; Torretta and Basilio, 2009). It produces fleshy drupes that are nearly orange when ripe, 4–10 mm long (Romanczuk, 1987; Berg and Dahlberg, 2001), and mainly dispersed by birds (Basilio *et al.*, 2006). The fruits of *C. ehrenbergiana* are consumed by the following species of birds: *Turdus amaurochalinus*, *Mimus saturninus*, *Pitangus sulphuratus*, *Saltator aurantiirostris*, *Thraupis sayaca*, *Pipraeidea bonariensis*, and *Elaenia parvirostris* (de la Peña and Pensiero, 2003; de la Peña, 2011; Ponce *et al.*, 2012). In the study area, *C. ehrenbergiana* is an important source of nourishment, as it is one of few species fruiting in the drier months, including the beginning of winter (Murriello *et al.*, 1993).

### Field sampling

Four 10-hectare plots separated by at least 200 m were mapped with GoogleEarth images using ArcGis 9.3 (ESRI, 2008). In each plot, 4–10 points at least 20 m apart were chosen at

random (using the ‘Create Random Points’ command in ArcGis). In the field, the nearest tree to each point was selected as that point’s focal tree. Fieldwork was carried out in February–March 2013, during peak fruiting in the study area (Murriello *et al.*, 1993). We observed bird behaviour at each focal tree for periods of three continuous hours within the first four hours after sunrise. Observers remained at least 10 m from the focal tree. During each observation period, bird species, the number of visits per hour (visitation rate), and the number of fruits consumed at each focal tree were recorded. We considered a visit legitimate if a bird completed its foraging bout by eating at least one fruit. We also recorded fruit-handling behaviour of birds. A bird species was considered a seed disperser (potentially a legitimate one) if it swallowed the whole fruit, or a pulp feeder if it pecked the fruit and discarded both the seed and the remaining pulp.

### Measurement of fruit display traits

In a randomly selected 0.125 m<sup>3</sup> area within the canopy, we directly counted the number of ripe fruits. The total number of fruits by tree (i.e. fruit crop) was estimated by extrapolating the number of fruits inside the cube to the total volume of the tree canopy. The canopy volume was estimated by the ellipsoid equation,  $V = (4/3) \pi abc$ , where  $V$  is the volume, and  $a$ ,  $b$ , and  $c$  are the three longest orthogonal semi-axes of the canopy (Sobral *et al.*, 2010).

At the end of our focal tree observations, 9–31 ripening fruits per tree were collected at random (based on availability). For each fruit, fresh weight and seed fresh weight were measured in the field with an Ohaus scale to the nearest 0.01 g. Fresh pulp weight was estimated as fruit fresh weight minus seed fresh weight. The diameter was measured on scaled photographs of the fruits using the software ImageJ 1.4g (Rasband, 1997). Photographs of fruits were taken with a Canon EOS 550D digital camera (12.0 megapixels). Total sugar concentration per individual fruit was measured in the field to the nearest 0.25 °Brix with a Reichert hand-held refractometer (0–50 °Bx). Sugar concentration was considered a surrogate of fruit quality (Smith *et al.*, 2007; Schaefer and Braun, 2009).

Because it was impossible to weigh individual fruits from trees with the same scale used for seeds, we estimated individual fresh weight to characterize individual pulp-related plant traits. To do so, we collected a subsample of 120 fruits and measured individual fruit and seed weight in the laboratory with an Ohaus Discovery 114C scale to the nearest 0.1 mg. We estimated individual fruit weight using linear regression between diameter (cm) and weight (g) of the subsample of fruit (estimated weight =  $-0.589 + 0.108 \times$  observed fruit diameter;  $r^2 = 0.828$ ,  $P < 0.0001$ ). Then, we estimated pulp fresh weight per fruit as estimated individual fruit weight minus individual observed seed weight. The pulp-to-seed ratio per fruit was estimated as individual pulp weight (as indicated above) divided by individual seed weight.

### Dispersed seed measurements

Between 12 and 22 March, 5–63 fruits at 18 sampled points were randomly collected from the ground (based on availability). Only those fruits showing evidence of bird consumption, such as pulp mashing or pecking, were collected. To avoid collecting seeds potentially dispersed in the previous year, only fresh-like seeds or seeds with attached fresh pulp were collected.

## Data analysis

### *Correlation matrix*

Fruit diameter was used as a surrogate of fruit size, since mean fruit fresh weight per tree and mean fruit diameter per tree were highly correlated ( $r = 0.919$ ,  $n = 24$ ,  $P < 0.0001$ ). Pearson correlations were run to explore the relationship among traits. For correlations, fruit crop was log-transformed due to a right-skewed distribution. Sequential Bonferroni corrections were applied to control the group-wide type I error rates at  $\alpha = 0.05$  (Rice, 1989).

### *Variance components*

To estimate the magnitude of variation within plants with respect to the magnitude of variation between plants, we applied analysis of variance components. The ratio between the variance component and the sum of variance components indicates the proportional contribution of the variation due to each level (Crawley, 2007). We applied a linear mixed model fitted by restricted maximum likelihood, with the corresponding trait as the response variable and plant as a random factor.

### *Phenotypic selection analyses*

Selection analyses follow Lande and Arnold (1983). We estimated the pattern and strength of bird-mediated phenotypic selection on fruit crop (unique measure), mean and CV of fruit traits (individual-level measures; diameter, pulp-to-seed ratio, sugar concentration). Individual relative fitness was estimated as the individual fitness measure divided by the population mean fitness. Fruit display traits were standardized. To analyse phenotypic selection on maternal traits, the bird visitation rate (individuals per hour) was used as the measure of fitness. This measure was used instead of the fruit consumption rate since the former has been typically used as a surrogate of seeds dispersed per plant (Howe and De Steven, 1979; Herrera and Jordano, 1981; Cordeiro and Howe, 2003; but see Brodie *et al.*, 2009). To analyse phenotypic selection on offspring traits, seed dispersal success (dispersed or non-dispersed seed) was used as the measure of fitness (Martínez *et al.*, 2007; Sobral *et al.*, 2010). Seeds in fallen fruits were considered as dispersed seeds, whereas those in fruits on trees were considered as non-dispersed seeds.

The directional selection differential ( $S_i$ ) was estimated as the covariance between relative fitness and each standardized trait. The disruptive/stabilizing selection differential ( $C_{ii}$ ) was estimated as the covariance between relative fitness and the squared deviation of each standardized trait (Brodie *et al.*, 1995). For the multivariate selection approach, only directional selection gradients ( $\beta_i$ ) were estimated due to power restrictions encountered for sample size. Two models were run to estimate selection gradients, one for mean and the other for sub-individual variation in fruit display traits. Because residuals of regression models departed from normality (Shapiro-Wilk  $W = 0.705\text{--}0.889$ ,  $n = 24$ ,  $P < 0.01$ ), the significance of differentials and selection gradients was estimated using generalized linear models (Nelder and Wedderburn, 1972). Significant linear gradients indicate that selection favours either a phenotypic mean increase (if positive) or decrease (if negative). For selection on maternal traits, we used Poisson error structure, a log link function, and penalized quasi-likelihood to account for overdispersion (Zuur *et al.*, 2009). For selection on offspring traits (0 = non-dispersed seed, 1 = dispersed seed), we used binomial error structure with a logit link function (Janzen and Stern, 1998; Sobral *et al.*, 2010). All analyses were run in R v.3.0.1 (R Development Core Team, 2013). The variance components analysis was run with the package lme4 [formula: fruit

trait  $\approx 1 + (1 | \text{plant})$  (Bates *et al.*, 2013)]. (Databases and scripts for selection analyses at [evolutionary-ecology.com/data/2873Appendix.pdf](http://evolutionary-ecology.com/data/2873Appendix.pdf))

## RESULTS

### *Characterization of fruit display and relationship between fruit crop and fruit quality*

Descriptive statistics for fruit display traits and the correlation matrix among individual fruit traits are given in Table 1 and Table 2, respectively. We detected no significant relationships between fruit crop and fruit quality (mean traits:  $r = -0.074$  to  $0.488$ ,  $n = 24$ ,  $P = 0.51-0.99$ ; sub-individual variation in traits:  $r = -0.452$  to  $0.298$ ,  $n = 24$ ,  $P = 0.79-0.99$ ). This indicates that larger fruit crops do not preclude a decrease in fruit quality.

### *Magnitude of sub-individual variation*

Variance component analysis showed that the magnitude of explained sub-individual variation was relatively high to intermediate: 57.08% for seed weight (variance component between plants = 0.00008, within plants = 0.00006), 49.45% for fruit diameter (variance component between plants = 0.397, within plants = 0.406), 48.10% for sugar concentration

**Table 1.** Descriptive statistics of fruit display traits in a *Celtis ehrenbergiana* population ( $n = 24$  plants)

Trait	mean $\pm$ s.d.
Fruit crop size	45752 $\pm$ 72395
Mean fruit diameter (cm)	8.517 $\pm$ 0.649
Mean fruit weight (g)	0.343 $\pm$ 0.072
Mean seed weight (g)	0.047 $\pm$ 0.008
Mean pulp-to-seed ratio	6.302 $\pm$ 1.952
Mean sugar concentration ( $^{\circ}$ Bx)	22.517 $\pm$ 3.111
CV fruit diameter	7.269 $\pm$ 1.706
CV fruit weight	20.721 $\pm$ 5.908
CV seed weight	18.445 $\pm$ 4.194
CV pulp-to-seed ratio	22.167 $\pm$ 5.862
CV sugar concentration	13.885 $\pm$ 6.046

Note: CV = coefficient of variation.

**Table 2.** Pearson product-moment correlation matrix of individual fruit display traits in a *Celtis ehrenbergiana* population ( $n = 616$  fruits)

	Sugar concentration	Pulp weight	Seed weight	Pulp-to-seed ratio
Fruit diameter	-0.330	0.993	0.450	0.560
Sugar concentration		-0.373	0.200	-0.494
Pulp weight			0.342	0.645
Seed weight				-0.430

Note: All coefficients,  $P < 0.001$ .

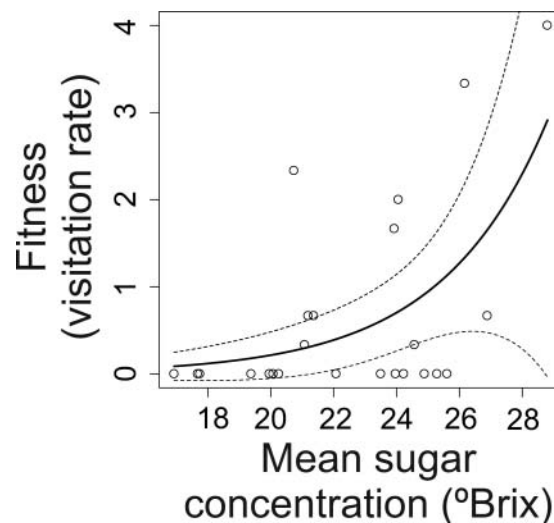
(variance component between plants = 8.563, within plants = 9.239), and 34.26% for pulp-to-seed ratio (variance component between plants = 1.950, within plants = 3.741).

#### Bird observations

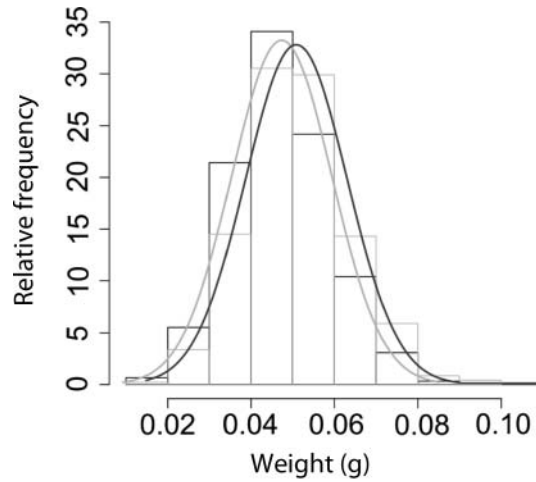
We recorded eight bird species that visited focal trees of *C. ehrenbergiana* in 72 hours of observation. These were *Turdus rufiventris*, *Mimus saturninus*, *Zonotrichia capensis*, *Agelaioides badius*, *Paroaria coronata*, *Pitangus sulphuratus*, *Elaenia parvirostris*, and *Icterus cayanensis*. We also recorded 48 visits (mean =  $0.667 \pm 1.146$  individual birds per hour) and 64 bird consumption events (mash, peck or swallow; mean =  $0.889 \pm 1.467$  fruits per hour). Pulp consumers (*Agelaioides badius*, *Zonotrichia capensis*, and *Paroaria coronata*) accounted for 70.8% of visits, whereas legitimate dispersers (*Turdus rufiventris*, *Mimus saturninus*, *Pitangus sulphuratus*, *Elaenia parvirostris*, and *Icterus cayanensis*) accounted for 29.2% of visits.

#### Bird-mediated phenotypic selection

At the maternal level, selection analyses showed positive directional selection on mean sugar concentration (Table 3; Fig. 1). The same pattern was observed for directional selection gradients (Table 4). In contrast, we found no selection on fruit crop, mean or coefficient of variation of fruit diameter or pulp-to-seed ratio (Tables 3 and 4). At the offspring level, we found positive directional selection on seed weight ( $S_i = 0.163 \pm 0.034$ ,  $n = 1091$ ,  $P < 0.0001$ ; Fig. 2), with an increase of 8.5% for this trait in the dispersed population, whereas stabilizing/disruptive selection was non-significant ( $C_{ii} = 0.008 \pm 0.022$ ,  $P = 0.708$ ).



**Fig. 1.** Bird-mediated directional selection on mean sugar concentration in a *Celtis ehrenbergiana* population ( $n = 24$  plants). Solid line depicts the fit from generalized linear models (see Methods). Dashed lines indicate  $\pm 1$  standard error.



**Fig. 2.** Bird-mediated directional selection on seed weight. Histograms with normal adjusted curves indicate the distribution for non-dispersed seeds (grey line, mean = 0.047, standard deviation = 0.012,  $n = 616$ ) and dispersed seeds (black line, mean = 0.051, standard deviation = 0.012,  $n = 475$ ) in a *Celtis ehrenbergiana* population.

**Table 3.** Standardized directional selection differentials ( $S_i$ ) and stabilizing/disruptive selection differentials ( $C_{ii}$ ) for fruit display traits in a *Celtis ehrenbergiana* population ( $n = 24$  plants)

Trait	Visitation rate (individual birds per hour)	
	$S_i \pm \text{s.e.}$	$C_{ii} \pm \text{s.e.}$
Fruit crop	$0.247 \pm 0.363$	$-0.038 \pm 0.146$
Mean fruit diameter	$-0.167 \pm 0.365$	$-0.211 \pm 0.258$
Mean pulp-to-seed ratio	$-0.335 \pm 0.360$	$-0.109 \pm 0.139$
Mean sugar concentration	<b><math>0.840 \pm 0.320</math></b>	$0.498 \pm 0.322$
CV fruit diameter	$-0.021 \pm 0.367$	$0.207 \pm 0.406$
CV pulp-to-seed ratio	$-0.049 \pm 0.366$	$0.316 \pm 0.319$
CV sugar concentration	$0.015 \pm 0.367$	$0.039 \pm 0.238$

*Note:* **Bold** font indicates significant differential,  $P = 0.0154$ . CV = coefficient of variation.

## DISCUSSION

Bird-mediated selection was found to act on fruit display traits in the studied population of *Celtis ehrenbergiana*. Selection pressures exerted by frugivores on maternal and offspring plant traits were uncoupled, which supports the idea that the relative fitness of an individual seed is not necessarily correlated with the relative fitness of its maternal phenotype (Jordano, 1995; Herrera, 2009).

Among maternal-level traits, mean sugar concentration was the main target of bird-mediated selection, which might be explained by fruit-handling behaviour. Mashers or pecking bird species (*Agelaioides badius*, *Zonotrichia capensis*, and *Paroaria coronata*)



**Table 4.** Directional selection gradients ( $\beta_i \pm$  s.e.) for fruit display traits in a *Celtis ehrenbergiana* population ( $n = 24$  plants)

Trait	Visitation rate (individual birds per hour)	
	mean	CV
Fruit crop	0.139 $\pm$ 0.350	0.331 $\pm$ 0.440
Fruit diameter	0.199 $\pm$ 0.442	0.141 $\pm$ 0.509
Pulp-to-seed ratio	0.269 $\pm$ 0.508	0.053 $\pm$ 0.499
Sugar concentration	<b>1.088 <math>\pm</math> 0.441</b>	-0.200 $\pm$ 0.418

*Note:* **Bold** font indicates significant differential,  $P = 0.031$ . CV = coefficient of variation.

accounted for most visits, for which sugar concentration translates into a key trait to evaluate reward quality (Levey, 1987). It is known that fine-tuned tasting abilities in birds respond to variations in sugar concentration of 1–2% (Levey, 1987; Schaefer *et al.*, 2003). In the population studied, mean variation in sugar concentration among plants was around 18%, a relatively high amount that allowed detection and discrimination by bird frugivores. However, we failed to detect selection on sub-individual variation in traits. The fact that within-plant variation was high or similar to between-plant variation suggests that there may be constraints on the response to selection exerted by frugivores (Jordano, 1987). Herrera (2009) suggested that selection on sub-individual variation might be more frequent than selection on trait means, a hypothesis currently supported in two species, *Phillyrea latifolia* (Herrera, 2009) and *Crataegus monogyna* (Sobral *et al.*, 2010, 2013a). In contrast, our results suggest that in this selection episode, plant-level unique traits and fruit trait means represented a first-level characteristic detected by frugivorous birds, while sub-individual variation represented an underlying level of selection (Sallabanks, 1993).

Bird-mediated selection also promoted larger seeds. Since birds could seldom discriminate individual seeds without tasting fruits, selection on seed size might have been a by-product of indirect selection through a correlation with maternal traits, a hypothesis that deserves further research (for complementary explanations, see Galetti *et al.*, 2013). We found a weak positive relationship between sugar concentration and seed weight (Table 2), suggesting that bird-mediated selection might promote more concentrated fruits, indirectly favouring larger seeds. From the plant's perspective, investment in mean quality of reward might be linked to a higher investment in offspring quality, which would translate indirectly into larger seed size (Martínez *et al.*, 2007). The evolutionary stability of this correlation would become crucial, since frugivores would not be able to predict seed size to counteract selection pressures against seed load. Alternatively, frugivores might face a conflict of interest between sugar concentration and seed load, where birds prioritize sugar concentration. While birds exert selection pressures on reward-related traits (maternal stage) regardless of seed handling behaviour, plants obtain a benefit at the offspring stage via a larger seed size. Moreover, the uncoupling of selection pressures might result in scenarios that promote the evolutionary stability of seed-dispersal mutualisms.

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### REFERENCES

- Agrawal, A.A., Conner, J.K. and Rasmann, S. 2010. Tradeoffs and negative correlations in evolutionary ecology. In *Evolution after Darwin: The First 150 Years* (G. Bell, W.F. Eanes, D.J. Futuyma and J.S. Levinton, eds.), pp. 243–268. Sunderland, MA: Sinauer Associates.
- Alcántara, J.M., Rey, P.J., Valera, F., Sánchez-Lafuente, A.M. and Gutiérrez, J.E. 1997. Habitat alteration and plant intra-specific competition for seed dispersers: an example with *Olea europaea* var. *sylvestris*. *Oikos*, **79**: 291–300.
- Avery, M.L., Goocher, K. and Cone, M.A. 1993. Handling efficiency and berry size preferences of cedar waxwings. *Wilson Bull.*, **105**: 604–611.
- Basilio, A.M. and Torretta, J.P. 2006. Polinización en el talar de Magdalena. In *Talares Bonaerenses y su Conservación* (E. Mérida and J. Athor, eds.), pp. 106–116. Buenos Aires: Fundación de Historia Natural ‘Félix de Azara’.
- Basilio, A.M., Torretta, J.P., Pereira, G.D. and Achaval, B. 2006. Red trófica en el Parque Costero del Sur. In *Talares Bonaerenses y su Conservación* (E. Mérida and J. Athor, eds.), pp. 138–148. Buenos Aires: Fundación de Historia Natural ‘Félix de Azara’.
- Bates, D., Maechler, M., Bolker, B. and Walker, S. 2013. *lme4: Linear Mixed-effects Models Using Eigen and S4*. R package version 1.0–5 [CRAN.R-project.org/package = lme4].
- Berg, C.C. and Dahlberg, S.V. 2001. A revision of *Celtis* subg. *Mertensia* (Ulmaceae). *Brittonia*, **53**: 66–81.
- Brodie, E.D., Moore, A.J. and Janzen, F.J. 1995. Visualizing and quantifying natural selection. *Trends Ecol. Evol.*, **10**: 313–318.
- Brodie, J.F., Helmy, O.E., Brockelman, W.Y. and Maron, J.L. 2009. Functional differences within a guild of tropical mammalian frugivores. *Ecology*, **90**: 688–698.
- Cabrera, A.L. 1971. Fitogeografía de la República Argentina. *Bol. Soc. Argentina de Botánica*, **14**: 1–42.
- Cagnoni, M.C., Faggi, A.M. and Ribichich, A.M. 1996. La vegetación de la Reserva ‘El Destino’ (Partido de Magdalena, Provincia de Buenos Aires). *Parodiana*, **9**: 25–44.
- Cordeiro, N. and Howe, H.F. 2003. Forest fragmentation severs mutualism between seed dispersers and an endemic African tree. *Proc. Natl. Acad. Sci. USA*, **100**: 14052–14056.
- Crawley, M.J. 2007. *The R Book*. Chichester: Wiley.
- Cueto, V.R. and López de Casenave, J. 2000a. Seasonal changes in bird assemblages of coastal woodlands in east-central Argentina. *Stud. Neotrop. Fauna E.*, **35**: 173–177.
- Cueto, V.R. and López de Casenave, J. 2000b. Bird assemblages of protected and exploited coastal woodlands in east-central Argentina. *Wilson Bull.*, **112**: 395–402.
- de la Peña, M.R. 2011. Observaciones de campo en la alimentación de las aves. *Biológica*, **13**: 1–88.
- de la Peña, M.R. and Pensiero, J.F. 2003. Contribución de la flora en los hábitos alimentarios de las aves en un bosque del centro de la provincia de Santa Fe, Argentina. *Ornitol. Neotrop.*, **14**: 499–513.
- ESRI. 2008. *ArcGis 9.3*. Redlands, CA: Research Institute (ESRI).
- Galetti, M., Guevara, R., Côrtes, M.C., Fadini, R., Von Matter, S., Leite, A.B. *et al.* 2013. Functional extinction of birds drives rapid evolutionary changes in seed size. *Science*, **340**: 1086–1090.

- Goya, J.F., Placci, G., Arturi, M.F. and Brown, A. 1992. Distribución y características estructurales de los talares de la reserva de la biosfera 'Parque Costero del Sur'. *Revista de la Facultad de Ciencias Agrarias (La Plata)*, **68**: 53–64.
- Hampe, A. 2001. The role of fruit diet within a temperate breeding bird community in southern Spain. *Bird Study*, **48**: 116–123.
- Hedge, S.G., Ganeshiah, K.N. and Uma Shaanker, R. 1991. Fruit preference criteria by avian frugivores: their implications for the evolution of clutch size in *Solanum pubescens*. *Oikos*, **60**: 20–26.
- Herrera, C.M. 2009. *Multiplicity in Unity: Plant Subindividual Variation and Interaction with Animals*. Chicago, IL: University of Chicago Press.
- Herrera, C.M. and Jordano, P. 1981. *Prunus mahaleb* and birds: the high-efficiency seed dispersal system of a temperate fruiting tree. *Ecol. Monogr.*, **51**: 203–218.
- Howe, H.F. 1981. Dispersal of a neotropical nutmeg (*Viola sebifera*) by birds. *Auk*, **98**: 88–98.
- Howe, H.F. and De Steven, D. 1979. Fruit production, migrant bird visitation, and seed dispersal of *Guarea glabra* in Panama. *Oecologia*, **39**: 185–196.
- Howe, H.F. and Estabrook, G.F. 1977. On intra-specific competition for avian dispersers in tropical trees. *Am. Nat.*, **111**: 817–832.
- Hunziker, A.T. and Dottori, N.M. 1976. Contribución al conocimiento sobre los talas (Celtis, Ulmaceae) de Argentina, con especial referencia a la región mediterránea. *Kurtziana*, **9**: 103–140.
- Izhaki, I. 2002. The role of fruit traits in determining fruit removal in east Mediterranean ecosystems. In *Seed Dispersal and Frugivory: Ecology, Evolution and Conservation* (D.J. Levey, W.R. Silva and M. Galetti, eds.), pp. 161–175. Wallingford, Oxon: CAB International.
- Janzen, F.J. and Stern, H.S. 1998. Logistic regression for empirical studies of multivariate selection. *Evolution*, **52**: 1564–1571.
- Jordano, P. 1982. Migrant birds are the main seed dispersers of blackberries in southern Spain. *Oikos*, **38**: 183–193.
- Jordano, P. 1987. Avian fruit removal: effects of fruit variation, crop size, and insect damage. *Ecology*, **68**: 1711–1723.
- Jordano, P. 1995. Frugivore-mediated selection on fruit and seed size: birds and St. Lucie's cherry, *Prunus mahaleb*. *Ecology*, **76**: 2627–2639.
- Jordano, P. 2000. Fruits and frugivory. In *Seeds: The Ecology of Regeneration in Natural Plant Communities* (M. Fenner, ed.), pp. 125–165. Wallingford, Oxon: CAB International.
- Jordano, P. and Schupp, E.W. 2000. Seed disperser effectiveness: the quantity component and patterns of seed rain for *Prunus mahaleb*. *Ecol. Monogr.*, **70**: 591–615.
- Lande, R. and Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution*, **37**: 1210–1226.
- Levey, D. 1987. Sugar tasting ability and fruit selection in tropical fruit-eating birds. *Auk*, **104**: 173–179.
- Marshall, D.J., Heppell, S.L., Munch, S.B. and Warner, R.R. 2010. The relationship between maternal phenotype and offspring quality: do older mothers really produce the best offspring? *Ecology*, **91**: 2862–2873.
- Martínez, I., García, D. and Obeso, J.R. 2007. Allometric allocation in fruit and seed packaging conditions the conflict among selective pressures on seed size. *Evol. Ecol.*, **21**: 517–533.
- Mesina, F.J. and Fox, C.W. 2001. Offspring size and number. In *Evolutionary Ecology: Concepts and Case Studies* (C.W. Fox, D.A. Roff and D.J. Fairbairn, eds.), pp. 113–127. New York: Oxford University Press.
- Mojonnier, L. 1998. Natural selection on two seed-size traits in the Common Morning Glory *Ipomoea purpurea* (Convolvulaceae): patterns and evolutionary consequences. *Am. Nat.*, **152**: 188–203.
- Murriello, S., Arturi, M. and Brown, A.D. 1993. Fenología de las especies arbóreas de los talares del este de la provincia de Buenos Aires. *Ecología Austral*, **3**: 25–31.

- Nelder, J.A.R. and Wedderburn, W.M. 1972. Generalized linear models. *J. R. Stat. Soc. A*, **135**: 370–384.
- Parodi, L. 1940. La distribución geográfica de los talares de la provincia de Buenos Aires. *Darwiniana*, **4**: 33–56.
- Ponce, A.M., Grilli, G. and Galetto, L. 2012. Frugivoría y remoción de frutos ornitócoros en fragmentos del bosque chaqueño de Córdoba (Argentina). *Bosque*, **33**: 33–41.
- R Development Core Team. 2013. R: *A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing [http://www.R-project.org/].
- Rasband, W. 1997. *ImageJ*. Bethesda, MD: National Institutes of Health [http://rsb.info.nih.gov/ij/].
- Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution*, **43**: 223–225.
- Romanczuk, M.C. 1987. Ulmaceae. In *Flora Ilustrada de Entre Ríos* (N.S. Troncoso de Burkart and N.M. Bacigalupo, eds.), pp. 15–21. Buenos Aires: Colección Científica Instituto Nacional de Tecnología Agropecuaria.
- Russo, S.E., Portnoy, S. and Augspurger, C.K. 2006. Incorporating animal behavior into seed dispersal models: implications for seed shadows. *Ecology*, **87**: 3160–3174.
- Sallabanks, R. 1993. Hierarchical mechanisms of fruit selection by an avian frugivore. *Ecology*, **74**: 1326–1336.
- Schaefer, H.M. and Braun, J. 2009. Reliable cues and signals of fruit quality are contingent on the habitat in black elder (*Sambucus nigra*). *Ecology*, **90**: 1564–1573.
- Schaefer, H.M., Schmidt, V. and Bairlein, F. 2003. Discrimination abilities for nutrients: which difference matters for choosy birds and why? *Anim. Behav.*, **65**: 531–541.
- Schupp, E.W. 1995. Seed–seedling conflicts, habitat choice, and patterns of plant recruitment. *Am. J. Bot.*, **82**: 399–409.
- Schupp, E.W., Milleron, T. and Russo, S.E. 2002. Dissemination limitation and the origin and maintenance of species-rich tropical forests. In *Seed Dispersal and Frugivory: Ecology, Evolution and Conservation* (D.J. Levey, W.R. Silva and M. Galetti, eds.), pp. 19–33. Wallingford, Oxon: CAB International.
- Smith, C.C. and Fretwell, S.D. 1974. The optimal balance between size and number of offspring. *Am. Nat.*, **108**: 499–506.
- Smith, S.B., McPherson, K.H., Backer, J.M., Pierce, B.J., Podlesak, D.W. and McWilliams, S.R. 2007. Fruit quality and consumption by songbirds during autumn migration. *Wilson J. Ornithol.*, **119**: 419–428.
- Snow, D.W. 1971. Evolutionary aspects of fruit eating by birds. *Ibis*, **113**: 194–202.
- Sobral, M., Larrinaga, A.R. and Guitián, J. 2010. Do seed-dispersing birds exert selection on optimal plant trait combinations? Correlated phenotypic selection on the fruit and seed size of hawthorn (*Crataegus monogyna*). *Evol. Ecol.*, **24**: 1277–1290.
- Sobral, M., Guitián, J., Guitián, P. and Larrinaga, A.R. 2013a. Selective pressures along a latitudinal gradient affects subindividual variation in plants. *PLoS One*, **8**: 1–7.
- Sobral, M., Guitián, J., Guitián, P. and Larrinaga, A.R. 2013b. Seed predators exert selection on the subindividual variation of seed size. *Plant Biol.* (DOI: 10.1111/plb.12118).
- Torres Robles, S.S. and Arturi, M. 2009. Variación en la composición y riqueza florística en los talares del Parque Costero del Sur y su relación con el resto de los talares bonaerenses. In *Parque Costero del Sur – Naturaleza, Conservación y Patrimonio Cultural* (J. Athor, ed.), pp. 104–121. Buenos Aires: Fundación de Historia Natural ‘Félix de Azara’.
- Torretta, J.P. and Basilio, A.M. 2009. Dispersión polínica y éxito reproductivo de cuatro especies arbóreas de un bosque xerófito de Argentina. *Rev. Biol. Trop.*, **57**: 283–292.
- Wheelwright, N.T. 1985. Fruit size, gape width and the diets of fruit eating by birds. *Ecology*, **66**: 808–818.
- Wheelwright, N.T. 1993. Fruit size in a tropical tree species: variation, preference by birds, and heritability. *Vegetatio*, **107/108**: 163–174.
- Zuur, A., Ieno, E.N., Walker, N.J., Saveliev, A.A. and Smith, G.M. 2009. *Mixed Effects Models and Extensions in Ecology with R*. New York: Springer.