On the adequacy of fruit removal as a proxy for fitness in studies of bird-mediated phenotypic selection

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Abstract

Premise: In fleshy-fruited plants, fruit removal is widely used as a proxy for plant reproductive success. Nevertheless, this proxy may not accurately reflect the number of seeds dispersed, an assumed better proxy for total fitness (fruit removal × mean number of seeds dispersed per fruit).

Methods: We examined under what circumstances fruit removal can be reliable as a proxy for total fitness when assessing bird-mediated selection on fruit traits. In

three populations of the Blue Passionflower (*Passiflora caerulea*), we used the number of fruits pecked per plant as a surrogate for fruit removal to estimate phenotypic selection on fruit and seed traits, and simulations of the effect of the fruit-seed number trade-off on the number of fruits removed.

Key results: Fruit removal was a good indicator of fitness, accounting for 55–68% of the variability in total fitness, measured as total number of seeds removed. Moreover, multivariate selection analyses on fruit crop size, mean fruit diameter and mean seed number using fruit removal as a fitness proxy yielded similar selection regimes to those using total fitness. Simulations showed that producing more fruits and a lower number of seeds per fruit can result in a negative relationship between fruit removal and total fitness.

Conclusions: Our results suggest that fruit removal can be reliably used as a proxy for total fitness when (1) there is a weak fruit number-seed number trade-off, (2) fruit crop size and fruit removal correlate positively, and (3) seed number variability does not largely exceed fruit number variability.

Keywords: bird frugivory; fitness components; fleshy fruits; natural selection; *Passiflora caerulea*; Passifloraceae, plant-animal interactions; seed dispersal; selection gradients

INTRODUCTION

Fitness is a pivotal, unifying concept of evolutionary biology, but at the same time, one of the most difficult to define (Endler, 1986; Ariew and Lewontin, 2004; Abrams, 2012). Although there is no single, coherent definition of fitness (Ariew and Lewontin, 2004), fitness has been broadly defined as the ability of an organism to survive, reproduce, and have descendants in future generations (Conner and

Hartl, 2004). More precisely, the absolute fitness of a biological entity is the number of offsprings an individual leaves in the next generation (Futuyma and Kirkpatrick 2017). Relative fitness, by contrast, is defined with respect to the population mean fitness, and it is what matters for natural selection to take place (Lande and Arnold 1983). In the context of animal-mediated seed dispersal, two major maternal fitness proxies have been commonly used to assess phenotypic selection on plant and fruit traits (Carr, 1992; Ortiz-Pulido et al., 2007; Sobral et al., 2013; Palacio and Ordano, 2018): (1) the number of fruits removed (i.e., fruit removal), and (2) the number of seeds removed (i.e., seed removal). More precisely, the number of seeds removed represents the offspring number completing the pre-dispersal stage and entering the dispersal stage (Alcántara and Rey, 2003).

By contrast, fruit removal is indirectly linked to seed dispersal, and it is often implicitly assumed that increased fruit removal entails increased seed delivery. Despite being widely used in eco-evolutionary studies, fruit removal has been disputed as a fitness proxy, given it often ignores the number of seeds effectively dispersed (Palacio et al., 2021). Nevertheless, there are several reasons for considering fruit removal as a fitness surrogate for seed removal. First, if fruit removal scales positively with seed removal, then fruit removal indirectly complies with the most common working definition of fitness in evolutionary biology, namely, the number of offspring produced over a selection episode (Arnold and Wade, 1984a; Conner and Hartl, 2004). Second, fruit removal is a sine qua non condition for plants to move further away from the mother plant, increasing the probabilities of seed survival and establishment (Janzen, 1970; Connell, 1971; Howe and Miriti, 2004; Schupp and Jordano, 2011). Therefore, increased fruit

removal is often translated into increased seed recruitment (Primack and Kang, 1989; Jordano and Herrera, 1995; Muñoz et al., 2017) and, consequently, fitness. If we assume that non-dispersed seeds do not contribute to a plant's fitness, the mere fact of having fruits removed irrespective of seed number, should confer these plants an advantage in terms of relative fitness over plants without fruit removal (Carr, 1992).

Third, both components are inherently linked, as fruits enclose seeds and fruits must be pecked or swallowed before seeds can be dispersed, so the contribution of fruit removal to plant fitness is expected to be significant (Nakashizuka et al., 1995; Izhaki, 2002; Snell et al., 2019). This is most evident in the case of species with a fixed number of seeds per fruit, for which the number of fruits removed often scales linearly with the number of seeds dispersed (e.g., Sobral et al., 2013; Palacio et al., 2020). By contrast, seed removal is expected to be more decoupled from fruit removal in multi-seeded species with a variable number of seeds per fruit (e.g., many species produce fruits that develop acropetally, where seed number per fruit changes drastically from bottom to top; Diggle, 1995) as the same number of fruits removed in two plants may lead to different numbers of seeds removed. This uncoupling is expected to occur if plants face trade-offs between producing more fruits and a low number of seeds per fruit (fruit number-seed number trade-off; Fig. 1). If such a trade-off is absent (constant or similar average seed number among plants), then increased fruit removal will increase seed removal, as seed number can be considered relatively constant (Fig. 1A). If a fruit number-seed number trade-off is present (Agrawal et al., 2010), then the product between fruit removal and average seed number may correlate positively or negatively with fruit removal, or show a hump-shaped relationship

(Fig. 1). For a given number of fruits removed, the possible relationships with fitness (fruit removal × mean number of seeds dispersed per fruit) will depend on both the strength of this trade-off and the variability in the number of seeds produced per fruit. If variability in seed number is relatively low, the relationship between fruit removal and seed removal will be positive (Fig. 1B; in the extreme case of no variation, this relationship is perfectly linear as in Fig. 1A). As variability in seed number increases, the right tail of the distribution reverses to a negative relationship, resulting in a hump-shaped pattern (Fig. 1C). Only when there is a strong fruit number-seed number trade-off, and a high variability in seed number, there will be a negative relationship between fruit removal and fitness (Fig. 1D). This is because the product between fruit removal and mean seed number per fruit of the plant with the lowest fruit removal must exceed that of the plant with the highest fruit removal. Given that the number of fruits is usually larger and more variable than the number of seeds per fruit (often in the order of hundreds or thousands), mean seed number per fruit of those plants with lowest fruit removal would have to be extremely high to supersede the effect of fruit removal. Therefore, fruit removal is expected to contribute more to fitness than the average number of seeds dispersed per fruit, and may explain why the relationship between fruit removal and fitness is usually positive (Murray, 1987; Carr, 1992; Palacio and Ordano, 2018).

However, no study has assessed whether the number of seeds can be approximated by the number of fruits removed in phenotypic selection studies of multi-seeded species. Therefore, our main question is whether and under what circumstances the number of fruits removed can be used as a fitness proxy for the number of seeds removed when assessing bird-mediated selection on fruit traits. To

this end, we first compared the effects of using fruit and seed removal as fitness proxies on selection regimes on fruit and seed traits in three populations of the Blue Passionflower (*Passiflora caerulea*). This species is particularly suitable for our goal, since pecked fruits remain in the plant, allowing better seed removal estimates than species whose fruits are ingested whole. This was done by estimating fruit removal as the number of pecked fruits, and seed removal (number of seeds dispersed) as the difference between the number of seeds (estimated from fruit diameter) and the number of remaining seeds in pecked fruits. Second, we derived a set of simulations testing how the strength of the fruit number-seed number trade-off would impact the relationship between fruit and seed removal. Three main outcomes would lead to the conclusion that fruit removal can be used as an adequate surrogate for seed removal: (1) there is no strong fruit-seed number trade-off per fruit (i.e., producing a large number of fruits does not entail producing a low number of seeds per fruit), (2) fruit and seed removal correlate positively, and (3) selection patterns are similar when using fruit or seed removal as fitness proxies.

METHODS

Study system

The Blue Passionflower *Passiflora caerulea* L. (Passifloraceae) is a vine, 2–10 m in height, native from southeastern Brazil, Bolivia, Paraguay, Uruguay, and Argentina (Killip, 1938). It is found in a wide diversity of habitat types, including rural, peri-urban and urban habitats. It produces large berries, orange when ripe, 43.1 ± 3.0 mm in length and 33.8 ± 2.0 mm in width (Bandeira et al., 2016). Each fruit encloses an average of 131.8 ± 72.2 seeds, 4.3 ± 0.16 mm in length, covered by red, fleshy appendages termed arils (Palacio et al., 2021). Fruits and seeds are

mainly consumed and dispersed by birds, including *Turdus rufiventris* (Turdidae),
Paroaria coronata (Thraupidae), *Zonotrichia capensis* (Passerellidae), *Saltator
aurantiirostris* (Thraupidae), *Thraupis sayaca* (Thraupidae) and *Tyrannus
melancholicus* (Tyrannidae) (Mendiondo and Amela García, 2006; Palacio et al.,
2021; Zaffignani and Palacio, 2021). Due to their large size, fruits are pecked, and
the beak is inserted into these to obtain the arilated seeds, and thus the diaspore is
represented by the bulk of seeds plus arils (Zaffignani and palacio, 2021; Fig. 2).
The same fruit is often pecked by the same individual, and multiple individuals
may peck the same fruit, with a variable number of seeds removed per peck, so that
the diaspore is represented by the bulk of seeds plus arils (Palacio et al., 2021). The
particularities of this system would allow better estimations of plant fitness than
other multi-seeded species, as pecked fruits remain in the plant (or eventually fall
to the ground), so the number of remaining seeds can be directly counted from the
fruit (see below). Due to these features, the number of fruits removed (fruit
removal *sensu stricto*) is not the same as the number of fruits pecked, and fruit
removal cannot strictly be quantified by definition. However, the number of fruits
pecked is a good surrogate for fruit removal in this system (Palacio et al., 2021),
and we equal fruit removal to the number of fruits pecked hereafter to extend our
theoretical ideas to other typical seed-dispersal mutualisms with small fruits.
Fruiting phenology is variable among populations and may extend all year round,
but has two flowering peaks (October-November and December-January) and
fruiting peaks (November-December and February-April) (Palacio et al., 2021).

Study area

The study was conducted in three cities from central and northern Argentina,
located at a minimum distance of 500 km. The plants at each site were thus

considered as three distinct biological populations. These cities comprise urban and peri-urban areas in which two major habitat types can be recognized: (a) built-up areas including a built-up city center and residential areas with numerous gardens and tree avenues, and (b) parks distributed over the study area composed mainly of mature trees.

(1) La Plata (34°55'S, 57°57'W, 26 m a.s.l., 719,013 inhabitants), Buenos Aires province (hereafter "La Plata"). This city is located in the pampas grasslands, 70 km from La Plata river shore; it has a densely built-up city center with tall buildings, parks regularly distributed throughout and residential areas in the surroundings with flat houses (Delucchi et al., 1993). The average annual rainfall is 993 mm, and the average annual temperature is 17.0 °C (SMN 2021).

(2) Santa Fe (31°38'S, 60°422W, 25 m a.s.l., 401,544 inhabitants), Santa Fe province (hereafter "Santa Fe "). It represents the 8th largest urban center from Argentina; it is located on the floodplains of the Salado and Paraná rivers. The average annual rainfall is 977 mm, and the average annual temperature is 18.5 °C (Cardozo et al., 2008).

(3) San Miguel de Tucumán (26°49'S, 65°13'W, 431 m a.s.l., 694,327 inhabitants), Tucumán province (hereafter "Tucumán"). It represents the largest urban center in subtropical Argentina; natural vegetation is dominated by subtropical montane forests (Grau and Kortsarz, 2012). It is located in the eastern foothills of the "Sierra de San Javier", a mountain range (500–1876 m a.s.l.), mostly dominated by "Yungas" semi-evergreen forests, located about 15 km west of the city. The average annual rainfall is 966 mm, and the average annual temperature is 19.3 °C (García et al., 2018).

Plant, fruit and seed trait measurements

We monitored 25 (La Plata), 29 (Santa Fe), and 20 (Tucumán) fruiting plants in three sampling visits (encompassing 1-7 sampling days per visit). Due to differences in flower and fruiting phenology between populations (Palacio et al., 2021), visits were adjusted to span the main fruiting peak (November-December) at approx. even intervals (Dec. 2020-Jan. 2021 in La Plata, Nov. 2020-Dec. 2021 in Santa Fe, and Oct. 2020-Feb. 2021 in Tucumán). At each sampling day, we recorded the number of ripe (orange) fruits (i.e., fruit crop size), the number of fruits with pieces of peel or pulp plus seeds removed (pecked fruits), and the number of fallen fruits. All the pecked and fallen fruits recorded were mature fruits.

To estimate the number of seeds removed per fruit ('seed dispersal'), we collected 1-10 (according to availability) fruits per plant in the first sampling visit (5 non-pecked and 5 pecked fruits), and 1-5 (according to availability) pecked fruits per plant in the two subsequent sampling visits (La Plata = 347 fruits, Santa Fe = 459 fruits, Tucumán = 235 fruits). For each fruit, we obtained the following measurements: ripening stage, degree of fruit pecking, fresh mass, fruit diameter, and seed number. Ripening stage was visually identified as unripe (fully green) or ripe (fully orange). The degree of fruit pecking was quantified as the percentage of peel removed (0%–intact fruit–, between 0 and 50% of the peel removed–slightly pecked fruit–, and greater than 50% of the peel removed–highly pecked fruit–). Fruit mass was measured with an Ohaus scale (nearest 0.1 g), and fruit diameter was measured from scaled photographs in ImageJ version 1.4 g (Schneider et al., 2012). In the case of pecked fruits (>50% of peel removed) whose diameter could not be measured directly, we assumed the contour of the fruit to have an ellipsoidal shape (see below).

Data analysis

To identify the presence of a phenotypic fruit number-seed number trade-off, we fitted a linear mixed model (LMM) between fruit crop size (predictor, fixed effect) and mean seed number per fruit (response), including the population as a random effect. To quantify the relationship between fruit crop size, fruit removal and seed removal, we fitted two separate generalized linear mixed models (GLMM): (1) a GLMM between (log) fruit crop size (predictor, fixed effect) and the number of pecked fruits per plant (response, negative binomial distribution, log-link function), including the plant nested within the population as random effect. And (2) a GLMM between the (log) number of pecked fruits (predictor, fixed effect) and the number of seeds removed (response, negative binomial distribution, log-link function), accounting for the plant nested within the population. In both models, the number of visits was included as a fixed effect. In the second model, we had two data per plant, because seed removal was estimated by subtracting the number of seeds removed between visits 2 and 1, and between 3 and 2. In all models, predictors were log-transformed to linearize the relationship between the response and the predictor.

For selection analyses, we considered two plant fitness proxies in the context of animal-mediated selection: (1) the number of pecked fruits per plant, and (2) the total number of seeds dispersed per plant. Both surrogates are dependent stages of seed dispersal (Wang and Smith, 2002), as fruits need to be first pecked to disperse at least one seed, and a non-pecked fruit results in no dispersal. Therefore, both fitness proxies assume that at least one seedling survives in the next generation, in contrast to non-pecked fruits, for which this probability is zero (Palacio et al., 2021). In addition, the overall number of seeds dispersed assumes

that every seed has the same probability to reach a suitable place for germination and growing, a factor largely dependent on bird behavior (Jordano and Schupp, 2000; Palacio et al., 2017b).

To estimate the number of seeds dispersed from pecked fruits (n_d), we first fitted a GLMM (negative binomial distribution, log-link function) between fruit diameter (predictor) and seed number (response) considering only intact fruits (n = 384 fruits from 74 plants). The plant nested within the population was included as a random factor. The variance explained by both fixed and random factors was substantially higher (conditional $R^2_{GLMM} = 0.84$) than the variance explained by fixed factors alone (marginal $R^2_{GLMM} = 0.43$), indicating better model fit when accounting for plant and population effects. We then counted the number of remaining seeds in pecked fruits (n_f) and estimated the initial number of seeds (n_i) by assuming an ellipsoidal shape, computing its minor axis (i.e., fruit diameter) and predicting the number of seeds with the GLMM. The difference between the estimated and the remaining number of seeds approximates the number of seeds dispersed per fruit ($n_d = n_i - n_f$), set to zero if $n_d < 0$. To scale this estimate to the plant level (S_p = number of seeds removed per plant), we averaged the number of seeds dispersed per fruit ($\bar{n}_d$) and multiplied this value by the number of fruits removed (f_r), $S_p = f_r \bar{n}_d$, thus defining a measure of total fitness (*sensu* Arnold and Wade, 1984b; see below). Given that fruit diameter in several pecked fruits (6.3% of slightly pecked fruits and 75.7% of highly pecked fruits) was estimated, rather than observed, we validated the ellipsoidal approach. To do this, we made four arbitrary image cuts (50%, 62%, 75% and 90%) on a random sample of intact fruits (50 fruits and 200 images per population), estimated the ellipse-derived diameter and relative bias (100×(observed diameter − predicted diameter)/predicted

diameter), correlated the predicted vs the observed diameter for each cut level, and

compared the regression line with 1:1 line as a reference (Johnson 2008; Appendix

S1; see the Supplementary Data with this article). We found strong correlations

between observed and predicted diameters (most r's > 0.90), low relative bias

(between 0.04 and 10%), and decreased precision with increased image cut level

(La Plata and Santa Fe from 5 to 10%, Tucumán from 8 to 20%) as expected

(Appendix S1). In Tucumán and Santa Fe, there was some level of diameter

underestimation for the 90% cut level and large fruits though. In other words, the

ellipsoidal approach yielded good fruit diameter estimates in those fruits with some

level of peel removal, with higher measurement variation in highly pecked fruits.

We estimated the strength and regime of bird-mediated natural selection on

fruit crop size, mean fruit diameter and mean seed number in the three Blue

Passionflower populations. To assess the direction and strength of selection on a

given trait independent of the indirect effect of other traits, we used a multivariate

selection approach (Lande and Arnold, 1983) following the protocol outlined in

Palacio et al. (2019) –Appendix S2. In the case of Tucumán, we excluded mean

seed number, due to its high correlation with mean fruit diameter (r = 0.86). If the

number of seeds dispersed per plant is taken as a measure of total fitness, the

number of pecked fruits (f_r) and the average number of seeds dispersed ($\bar{n}_d$) can be

seen as multiplicative components of fitness (*sensu* Arnold and Wade, 1984b):

Total fitness = $f_r \bar{n}_d$

$\bar{n}_d$ was estimated as the average in the mean number of seeds removed in

both time intervals (visit 2 – visit 1 and visit 3 – visit 2). Separate phenotypic

selection analyses were undertaken for each population and fitness measure (f_r and

total fitness); f_r was estimated as the (accumulated) number of pecked fruits recorded in the last visit (Palacio et al., 2021). Fitness components were relativized to their mean population values (De Lisle and Svensson, 2017), and traits were standardized to mean = 0 and variance = 1 before analyses. Significant linear gradients (β_i) indicate either a mean increase (if positive) or decrease (if negative) in fitness with increases in the value of a phenotypic trait (holding all other traits constant). Non-linear selection gradients were not estimated due to sample size constraints. Since normality assumptions for every model were not met (Appendix S2), standard error and 95% confidence intervals for selection gradients were estimated using 1000 bootstrap replicates (Palacio et al., 2019). Finally, we tested the contribution of fruit removal to the variability in total fitness, by fitting a linear model between (log) total fitness (response), the (log) number of fruits pecked and the (log) mean number of seeds removed per fruit per plant (predictors) (Rother et al., 2016), and computing the average R^2 for each predictor over orderings among predictors (LMG metric in the R package relaimpo; Grömping, 2006).

Although our study system is suitable to quantify the number of seeds effectively dispersed, this is not typical in the sense that most fleshy-fruited plants have small fruits that are often ingested whole. Therefore, to generalize our results to other seed-dispersal systems, we complemented our analysis with simulations to assess how the fruit-seed number trade-off influenced the relationship between fruit removal and total fitness. To do this, we first simulated fruit crop size values of a hypothetical plant population of 500 individuals creating a sequence of range 1–500 fruits. The assumptions were that (1) fruit removal correlates positively with fruit crop size ("fruit crop size hypothesis"; reviewed by Palacio and Ordano 2018), with the number of fruits removed following a binomial distribution with parameter

π = 0.7 (fixed for every iteration); and (2) the mean number of seeds produced is negatively related to fruit crop size, with the number of seeds removed following a Poisson distribution with parameter $\lambda = 100 \times \exp(\beta \times$ number of fruits removed). These β's took 10,000 evenly distributed values ranging between -0.2 and 0 to simulate the effects of varying degrees of the fruit number-seed number trade-off. At each iteration we fitted a generalized linear model (Poisson, log-link) between total fitness (fruit removal × seed removal) and fruit removal with a quadratic term to account for non-linearities of this relationship, and computed the coefficient of variation in seed removal.

All graphs and analyses were performed in R 4.0.2 (R Core Team, 2020), using the packages lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017), MuMIn (Barton, 2020), MVN (Korkmaz et al., 2014), car (Fox and Weisberg, 2019), boot (Canty and Ripley, 2020), dplyr (Wickham et al., 2020), reshape2 (Wickham, 2007), and ggplot2 (Wickham, 2016), ggdist (Kay 2021), and mgcv (Wood, 2017).

RESULTS

We recorded data on fruit traits and fruit removal of 74 plants taking into account the three sampling visits (n = 210 fruit removal data and n = 1041 fruits). A summary of the phenotypic traits measured in each population is shown in Table 1. We found considerable variation in terms of fruit and seed traits among populations. For instance, La Plata produced more than twice as many fruits compared to the two other populations, whereas Santa Fe had substantially larger fruits (an average of 6 mm wider) with more seeds (an average of 50-100 more seeds per fruit) than the two other populations (Table 1). When comparing the diameter of intact and pecked fruits within the same population (Fig. 3), we found

similar trait distributions, but pecked fruits were smaller than intact fruits in La Plata, and showed more variability in Santa Fe. We did not find a significant relationship between overall fruit production and mean seed number per fruit (LMM coefficient = 2.75, SE = 5.29, conditional R^2_{GLMM} = 0.44, n = 74, P = 0.60). In contrast, we found significant relationships between fruit crop size and fruit removal (GLMM coefficient = 0.72, SE = 0.07, conditional R^2_{GLMM} = 0.75, n = 210, P < 0.0001), and, to a lesser extent, between fruit removal and seed removal (GLMM coefficient = 1.99, SE = 0.41, conditional R^2_{GLMM} = 0.43, n = 65, P < 0.0001).

Regarding fitness components, fruit removal accounted for 55.1– 68.3% of the variability in total fitness (Table 2). Natural selection analyses showed different selection patterns between populations (Table 3, Fig. 4). For instance, we found positive directional selection on fruit crop size in La Plata through both the number of fruits removed and total fitness (Fig. 4A-B). In Tucumán, we found positive directional selection on mean fruit diameter through both fitness components (Table 3, Fig. 4C-D). In contrast, we found no significant selection on any trait in Santa Fe (Table 3). Overall, we detected consistently similar selection patterns in each population regardless of the fitness component used.

In addition, our simulations revealed that the fruit number-seed number trade-off influenced the relationship between fruit removal and total fitness (Fig. 5A). In particular, the relationship between the phenotypic trade-off (quantified as the linear coefficient between fruit crop size and mean number of seeds) and the fruit removal-fitness relationship (quantified as the quadratic coefficient between fruit removal and total seed number) was positive, whereas the relationship between the fruit removal-fitness relationship and the coefficient of variation was

negative (Fig. 5B). When inspecting some examples of the fruit removal-fitness relationship (Fig. 5C-F), we found that strong trade-offs translated into negative relationships between fruit removal and fitness (Fig. 5C) and, as the magnitude of the trade-off decreased, a hump-shaped pattern emerged (Fig. 5D-E). Finally, as the fruit number-seed number trade-off approaches zero, the relationship between fruit removal and fitness approaches a perfectly linear relationship (Fig. 5F). Overall, only when there is a strong fruit-seed number and high variability in seed number, there can be a negative relationship between fruit removal and fitness.

DISCUSSION

In fleshy-fruited plants, the number of fruits removed (fruit removal) is one fitness component in the progression of reproductive events from flowering to seedling establishment (Sargent, 1990; Wang and Smith, 2002). In animal-mediated seed dispersal systems, fruit removal has been often criticized as a proxy for fitness, because it neglects the number of seeds effectively delivered. However, its contribution to overall seed dispersal has received little attention in phenotypic selection studies. Our results show that fruit removal contributed significantly to total fitness (fruit removal × mean number of seeds removed per fruit) in three populations of a fleshy-fruited plant. In addition, multivariate selection analyses on a set of fruit traits (fruit crop size, mean fruit diameter and mean seed number) yielded consistently similar selection regimes when using either fruit removal or total fitness as fitness proxies in the three populations. These results support other ecologically focused studies which have used other fitness components. For instance, Herrera et al. (1994) and Muñoz et al. (2017) found that higher fruit removal by frugivores related positively to seedling density in a mast-fruiting plant of the Mediterranean forest and in 22 fleshy-fruited species in the Central Andes of

Colombia, respectively, suggesting that fruit removal can have positive effects on fitness even at fairly advanced stages of the seed dispersal loop.

Our simulations focused on the impact that the fruit-seed number trade-off had on the relationship between fruit removal and fitness irrespective of the seed-dispersal system analyzed, to assess the validity and generality of our results. We found that producing more fruits and a lower number of seeds per fruit influenced the relationship between fruit removal and total fitness. When the trade-off is weak, the relationship between fruit removal and total fitness is nearly linear, because the mean number of seeds per fruit can be considered constant (represented by the average seed number). As this trade-off becomes stronger and the variability in seed number increases, a hump-shaped pattern emerges. This is because as fruit removal increases, the number of seeds removed decreases to the point of reversing the positive pattern between fruit removal and total fitness. Only when there is a sharp fruit number-seed number trade-off (along with a high variability in seed number) the relationship between fruit removal and total fitness may eventually become negative. As stated above, these simulations assume that there is a positive relationship between fruit removal and fruit crop size (see below). Interestingly, to our knowledge, there are no cases in which fruit removal and total fitness are negatively related.

Overall, based on our simulations and selection analysis we suggest that three conditions should be met to use fruit removal as a surrogate for seed removal in studies of phenotypic selection: (1) there is no strong phenotypic trade-off between producing a large number of fruits and a low number of seeds per fruit, (2) fruit removal and fruit crop size correlate positively, and (3) seed number variability does not largely exceed fruit number variability. As fruit and seed

production are resource limited (Lee, 1988), condition (1) would be expected to be ubiquitous (Smith and Fretwell, 1974; Venable, 1992). However, since fruit crop size is determined by multiple factors, including resource availability (Wang et al., 2020), plant size (Minor and Kobe, 2019), and pollination service (Tamburini et al., 2019), negative phenotypic correlations between fruit and seed number has been more commonly found under experimental conditions than in nature (Palacio and Ordano, 2018). This suggests that fruit number and average seed number could genetically, rather than phenotypically, trade off (Agrawal et al., 2010). Condition (2) is a prevailing pattern among fleshy-fruited plants, as Palacio and Ordano (2018) found in a meta-analysis of the relationship between fruit crop size and bird fruit removal, and continues being supported by recent studies (e.g., Quitián et al., 2019; Ramaswami et al., 2019; Gopal et al., 2020; Palacio et al. 2021). Finally, condition (3) seems to be widely met among fleshy-fruited plants. For instance, numerous species have a constant number of seeds (e.g., around 80% out of the 320 species in the FRUBASE dataset; Jordano, 1995a), and thus this condition becomes trivial, as seed number variability is zero. In the case of multi-seeded species with a variable number of seeds, such as the Blue Passionflower, fruit crop size variation often exceeds seed number variation (e.g., coefficients of variation: 106.9–175.8% vs 43.1–77.0% in *Vassobia breviflora*, Palacio, 2017; 39.8% vs 32.2% in *Solanum lycopersicum*, Aralikati et al., 2018; 49.0% vs 38.4% in *Citrus grandis*, Hossain et al., 2018; 66.0% vs 37.2% in *Capsicum anuum*, Ridzuan et al., 2018). This supports the presumably more prominent role of fruit over seed production for plant fitness in a disperser-mediated selection context.

The phenotypic fruit-seed number trade-off observed in the three Blue Passionflower populations matched the expectations on the relationship between

fruit removal and total fitness derived from simulations (condition 1). In particular, fruit-seed number trade-offs were not detected in any of the populations (model coefficients of this trade-off ranging between -0.0005 and 0.001). Moreover, fruit removal and fruit crop size correlated positively (condition 2) in the three populations (r = 0.24–0.79). Both results led to positive linear relationships between fruit removal and fitness (similar to that in Fig. 5F) or exponentially positive (for the positive trade-off) relationships when parameterizing simulations with these empirical values (results not shown). Finally, variability in fruit production was significantly higher than variability in seed number (condition 3) in the three populations (Table 1). The fulfillment of these conditions in our study system ultimately translated into similar selection patterns on fruit and seed traits when using fruit and seed removal as fitness proxies. We also detected different selection patterns when using different fitness components in two populations. In particular, we detected positive selection on fruit crop and fruit size in La Plata and Tucumán, respectively. These results support previous studies which found phenotypic selection on different fruit traits, including fruit crop size and fruit size (Jordano 1995b; Sobral et al. 2010; Palacio et al. 2017b, 2021). Since fruit size does not impose morphological constraints on fruit consumption and entails a large amount of reward, it is expected that birds exert selection on larger fruits (Wheelwright, 1985; McFadden et al., 2022). In turn, as birds can presumably discriminate individual seeds after a fruit is torn, birds exert direct, rather than indirect, selection on seed number. Therefore, larger fruits would benefit not only birds by providing a higher reward, but also plants by increasing the number of seeds produced and dispersed. We note that we quantified selection on only three phenotypic traits, yet it is known that birds impose selection on others, including

color, nutrient content, and secondary metabolites (Ordano et al., 2017; Palacio et al., 2021; Nelson and Whitehead, 2021). Altogether, our results suggest that the number of fruits removed and total fitness could be more tightly coupled than previously thought, at least in certain populations.

Although the positive association between fruit removal and total fitness is relevant in the short term, this does not prevent conflicting selection from occurring in post-dispersal stages of plant recruitment (Schluter et al., 1991). We therefore stress that analyzing fitness components in isolation limits our ability to compare phenotypic selection on fruit and seed traits, because these may result in different selective outcomes that can be canceled out, or even reversed, in subsequent life stages of the seed dispersal cycle. For instance, Alcántara and Rey (2003) showed that conflicting selection pressures on seed size during and after dispersal reversed the negative pattern of selection exerted by birds in *Olea europaea*. Similarly, Martínez et al. (2007) found that selection imposed on larger seeds by birds was canceled by opposite selection pressures exerted by seed predators in *Crataegus monogyna*. Finally, non-dispersed seeds were subject to positive directional selection on seed size, whereas ant-dispersed seeds experienced stabilizing selection on seed size in *Helleborus foetidus* (Manzaneda et al., 2009). Although this evidence suggests that using different fitness components may lead to different selection regimes on fruit traits, the association between different fitness components is often not tested, highlighting the need of analyzing several fitness components in animal-mediated dispersal systems.

Our results support that, given a set of assumptions, fruit removal is often an adequate proxy for seed removal in phenotypic selection studies. Moreover, our results have practical consequences for estimating plant fitness in the field, e.g., in

a large number of individuals or in species with short phenologies. A major future direction should be to validate our simulation model in other seed-dispersal systems, as well as to experimentally test the effect of different bird behaviors on fruit and seed removal.

ACKNOWLEDGEMENTS

AUTHOR CONTRIBUTIONS

F.X.P. and M.O. conceived and designed the research. F.X.P., J.F.C. and M.O. conducted fieldwork and image analyses. F.X.P. analyzed the data and wrote the initial draft of the paper. J.F.C., M.O. and D.M. made substantial edits to subsequent drafts of the manuscript.

DATA AVAILABILITY STATEMENT

All the data and associated R code to reproduce the main results of the paper are available at https://github.com/facuxpalacio/Passiflora_fitness.

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Table 1. Summary of plant and fruit traits (based on intact fruits) of three Blue

Passionflower (*Passiflora caerulea*) populations from Argentina. Mean ± SD

(sample size) is shown. CV: coefficient of variation.

Trait	La Plata	Santa Fe	Tucumán
Fruit crop size	69.85 ± 108.98 (75)	32.84 ± 27.42 (79)	29.09 ± 27.23 (76)
Number of pecked fruits	9.71 ± 19.08 (75)	8.81 ± 8.03 (79)	2.88 ± 4.32 (76)
CV fruit crop size	140.84 ± 13.58 (75)	119.09 ± 30.24 (79)	128.71 ± 17.16 (76)
Fruit diameter (mm)	32.16 ± 5.97 (114)	38.29 ± 4.38 (143)	32.47 ± 6.97 (141)
Fruit mass (g)	12.07 ± 5.85 (114)	19.07 ± 5.81 (143)	10.27 ± 6.49 (141)
Seed number per fruit	152.72 ± 79.73 (114)	197.06 ± 66.55 (143)	104.94 ± 73.07 (141)
CV seed number (%)	52.20 (114)	33.77 (143)	69.63 (141)
Number of seeds removed per fruit	71.44 ± 64.85 (114)	168.35 ± 183.24 (143)	59.48 ± 50.38 (141)

Table 2. Relative contribution of fitness components (fruit removal and mean

number of seeds removed) to variability in total fitness (fruit removal × mean

number of seeds removed per fruit per plant). R^2 partitioned by averaging over

orders (LMG metric) is shown.

Source of variation	La Plata	Santa Fe	Tucumán
Number of fruits removed	0.683	0.600	0.551
Mean number of seeds removed	0.223	0.398	0.336
Total (R^2)	0.907	0.998	0.888

Table 3. Linear multivariate selection on fruit traits (fruit crop size, mean fruit diameter and mean seed number) using two fitness estimators (number of pecked fruits and total number of seeds dispersed) in three *Passiflora caerulea* populations. Standardized directional selection gradients (β_i), and bootstrap standard errors (SE) are shown. Significant gradients are shown in bold. *: $P < 0.05$.

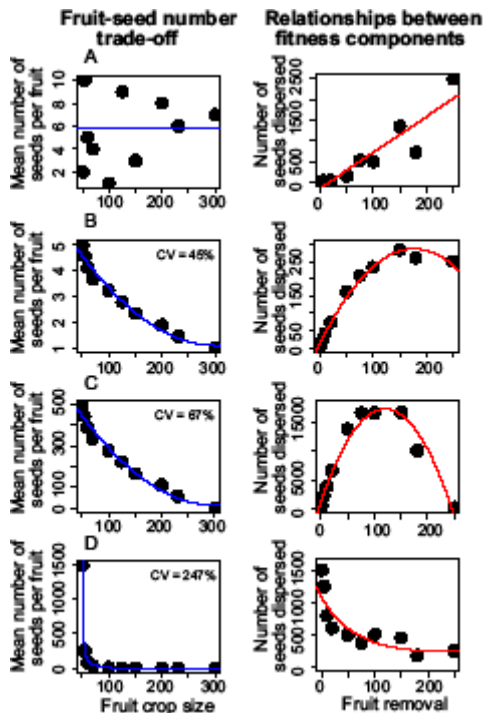| Fitness	Trait	La Plata		Santa Fe		Tucumán	
		β_i	SE	β_i	SE	β_i	SE
Number of pecked fruits	Fruit crop size	**1.26***	0.45	0.23	0.19	0.12	0.88
	Mean fruit diameter	−0.02	0.34	−0.01	0.08	**0.49***	0.26
	Mean seed number	0.11	0.30	−0.11	0.10	-	-
Total number of seeds dispersed	Fruit crop size	**2.28***	1.12	0.21	0.19	0.12	1.00
	Mean fruit diameter	−0.82	0.72	−0.07	0.12	**0.60***	0.31
	Mean seed number	0.92	0.65	0.10	0.16	-	-

Figures legends

Fig. 1. Influence of the fruit number-seed number trade-off and seed number variability on the relationship between fruit removal, seed removal and fitness. (A) If seed number per fruit is independent of fruit crop size (no trade-off, coefficient of variation = 0), and fruit removal relates positively to fruit crop size ('fruit crop size hypothesis'), then fruit removal will scale linearly with total fitness because it only depends on the number of fruits removed. As the relationship between seed number per fruit and fruit crop size becomes more negative with a concomitant increase in seed number variation (B-C), the relationship between fruit removal and fitness starts showing a hump-shaped pattern. Only when the trade-off is strong enough and seed number variation is high (D), the relationship between fruit removal and fitness can be reversed to a negative relationship.
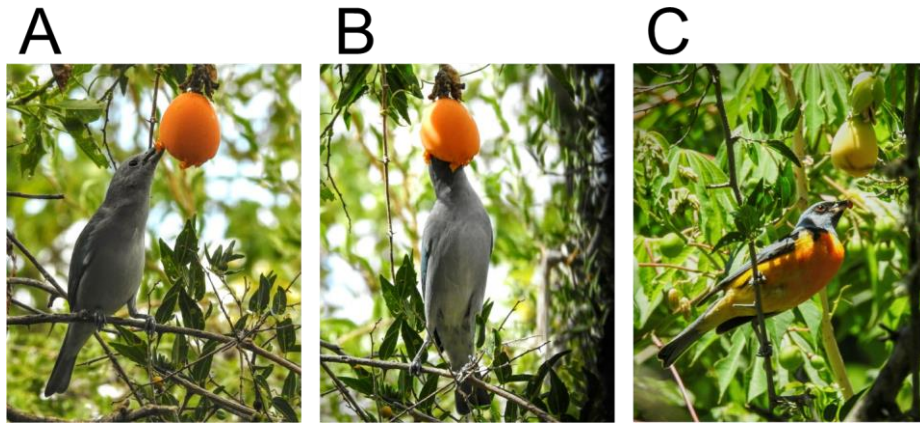
Fig. 2. Consumption of *Passiflora caerulea* fruits by birds. The peel is first bitten and torn off, and then the beak is inserted into the fruit to reach the arilated seeds. (a-b) Sayaca Tanager (*Thraupis sayaca*), (c) Blue-and-yellow Tanager (*Rauenia bonariensis*). Photo credits: Martín Lépez.
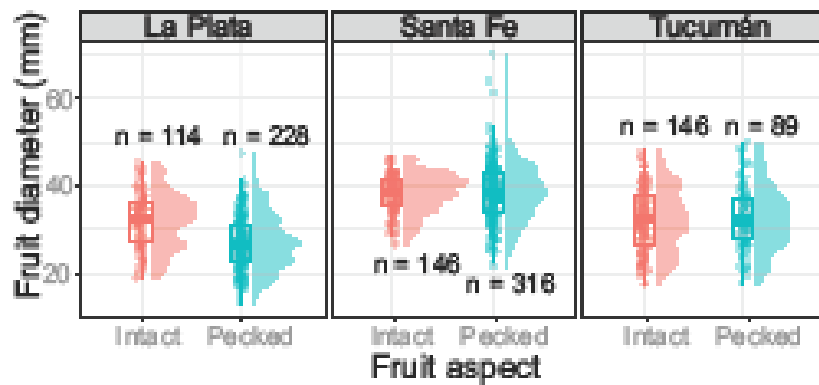

Fig. 3. Diameter (mm) distributions of pecked and entire fruits in three *Passiflora caerulea* populations. Boxplots and kernel distributions are shown. Points represent individual fruits. n: sample size.
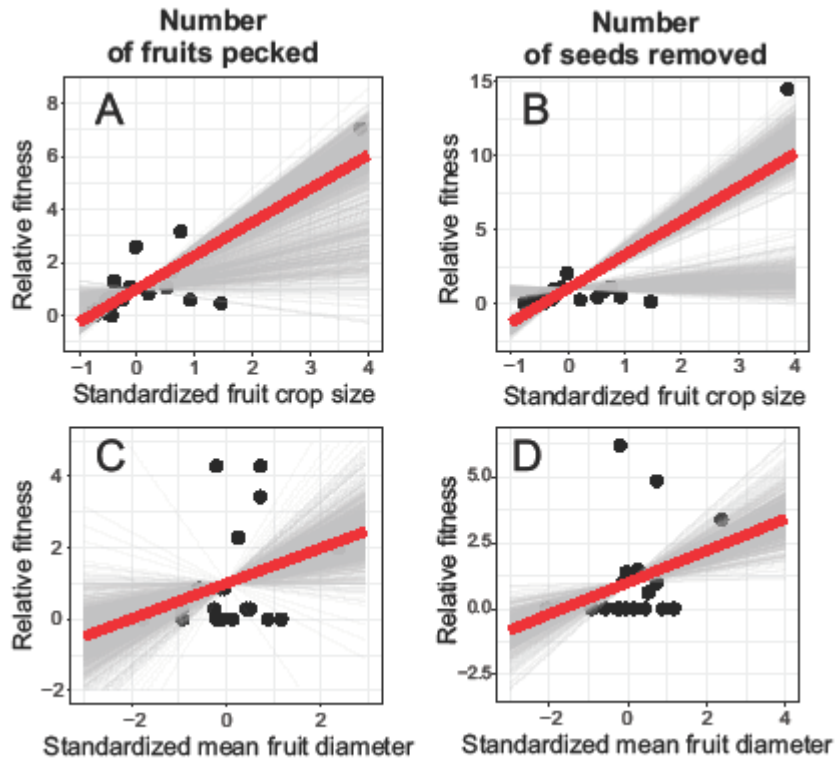
Fig. 4. Bird-mediated selection on fruit traits (fruit crop size, mean fruit diameter and mean seed number) using three fitness estimators (number of pecked fruits, mean number of seeds removed and total number of seeds removed) in three *Passiflora caerulea* populations (A-B: La Plata, C-D: Santa Fe). Black points represent observed values, red lines depict linear selection between standardized phenotypic traits and relative fitness conditional on the mean of the remaining phenotypic traits (z = 0), and gray lines depict 1000 bootstrap samples (see Methods).
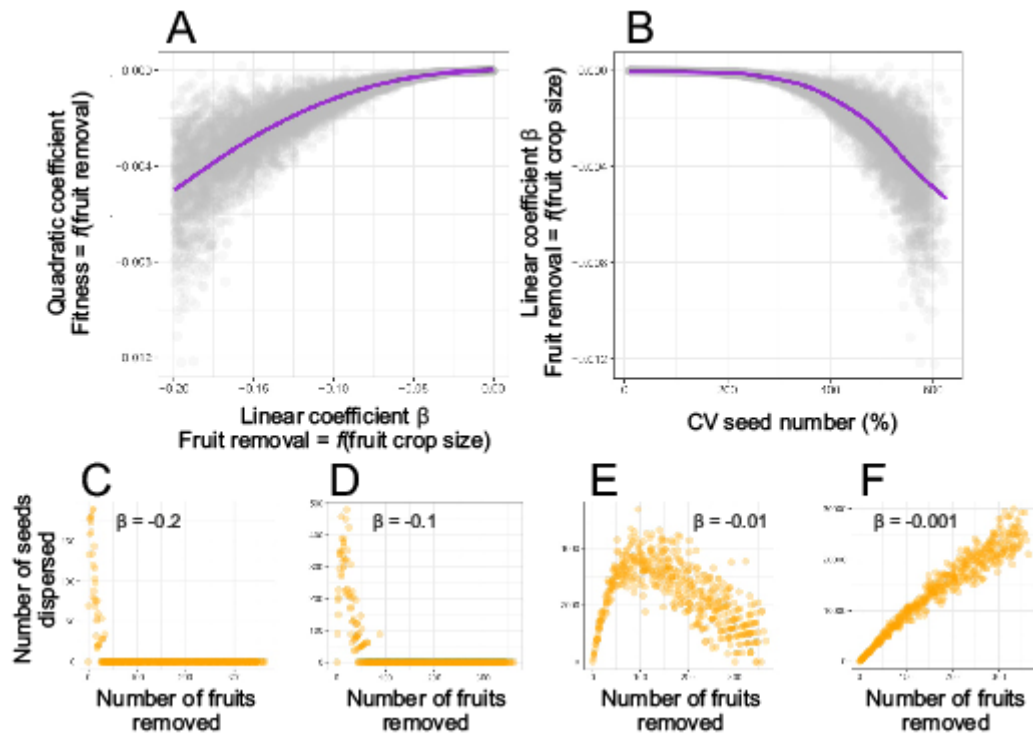
Fig. 5. Simulations of the effect of the fruit number-seed number trade-off between fruit removal and total fitness (number of fruits removed × mean seed number). (A) Relationship between the fruit number-seed number trade-off (linear coefficient derived from a model between fruit crop size and mean seed number) and fruit removal-fitness relationship (quadratic coefficient derived from a model between the number of fruits removed and total fitness). (B) Relationship between the coefficient of variation (CV) in seed number and fruit removal-fitness relationship. (C-F) Examples of fruit removal-fitness relationships for different values of the fruit number-seed number trade-off (β), which represent individual data points in (A). As the magnitude of fruit number-seed number trade-off and the variation in seed number decrease (i.e., approaches zero) the relationship between fruit removal and fitness changes from negative (C) to a hump-shaped pattern (D-E), and then to positive (F). Points represent simulated data, and lines represent fits of generalized additive models.