

Fruit sizes and the structure of frugivorous communities in a New Guinea lowland rainforest

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Abstract A community of frugivorous insects was studied by rearing of 25 565 individual insects representing three orders (Coleoptera, Lepidoptera and Diptera except Drosophilidae) from 326 woody plant species in a lowland rainforest in Papua New Guinea. Fruits from 19.3% of plant species were not attacked by any insect order, 33.4% of plant species were attacked by a single order, 30% by two orders and 17.2% by all three orders. The likelihood of attack by individual orders was positively correlated so that a higher proportion of plant species than expected suffered either no attack at all or was attacked by all three insect orders. Fruits from most of the plant species exhibited low rates of attack and low densities of insects. One kilogram of fruit was attacked on average by 11 insects, including three to four Coleoptera, six Diptera and one Lepidoptera. Thus, we reared on average one insect from 10 fruits, including one Diptera from 14 fruits, one Coleoptera from 22 fruits and one Lepidoptera from 100 fruits. Only 72 out of the 326 plant species hosted more than one insect per 10 fruits, and only seven species supported a density of greater than one insect per fruit. Our results suggest that specialized insect seed predators are probably too rare to maintain the diversity of vegetation by density-dependent mortality of seeds as suggested by the Janzen–Connell hypothesis. Fruit weight, fruit volume, mesocarp volume, seed volume and fleshiness had no significant effect on the probability that a fruit would be attacked by an insect frugivore. However, fruits attacked by Diptera were significantly larger and had larger volume of both mesocarp and seeds than fruits attacked by Coleoptera and Lepidoptera.

Key words: Coleoptera, Diptera, Lepidoptera, seed-eating herbivore, seed predation.

Insect frugivores may have an immediate impact on plants due to direct and indirect seed mortality (Moore 2001). They may reduce viability of the enclosed seeds (Janzen 1971; Herrera 1982) or open the way to subsequent attack by pathogens (Christensen 1972; Herrera 1982). Insect frugivores could be divided into groups of pre-dispersal and post-dispersal seed feeders (directly influencing mortality) and pulp feeders (possibly indirectly influencing mortality).

The main pre-dispersal seed predator groups include weevils (e.g. Janzen 1980; Lyal & Curran 2000, 2003; Nakagawa *et al.* 2003; Pinzon-Navarro *et al.* 2010), bruchids (e.g. Janzen 1980; Kergoat *et al.* 2005; Delobel & Delobel 2006), moths (e.g. Nakagawa *et al.* 2003; Hoddle & Hoddle 2008; Adamski *et al.* 2010; Brown *et al.* 2014) and some wasps (Janzen 1979; Weiblen 2002), bugs (Slater 1972) and katydids (Tan 2011). The most abundant post-dispersal frugivores are drosophilid flies (Mitsui *et al.* 2010) and sap beetles (Grimbacher *et al.* 2013), which are feeding on decaying mesocarp and participating in decomposition of the

fleshy pulp. Some bruchids also attack seeds only once the pulp is rotten or removed (e.g. by vertebrate frugivores; Moore 2001). There are also insect groups associated with fallen fruit, including carabid or staphylinid beetles, that are omnivorous, or predators attacking frugivores on fruits (Borcherding *et al.* 2000; Grimbacher *et al.* 2013). Frugivorous insects that do not directly affect seed mortality as they feed on pulp (i.e. mesocarp) include flies, particularly tephritids (e.g. Novotny *et al.* 2005; Copeland *et al.* 2006; Raga *et al.* 2011), and moths (McQuate *et al.* 2000).

Fruit size plays an important role in shaping the relationships of plants with both their predominantly vertebrate and invertebrate dispersers or consumers. Fruits may be attractive to herbivores because of their high-nutrient resources (Grubb *et al.* 1998); it remains unclear whether pulp evolved primarily as a reward for dispersers (Mack 2000) or a mechanical protection of the seed (Herrera 1982). Fleshy-fruited species have lower seed predation rates than dry fruited species (Janzen 1969; Herrera 1987; Wright 1990) as some seed predators, for instance bruchid beetles, cannot penetrate pulp in fleshy fruits. The fleshy-fruited species may therefore be colonized only after dehiscence (in case of

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dehiscent fruits) or when the pulp is damaged by vertebrate predators (Wright 1990) or decayed during the post-dispersal phase. However, pulp size and composition also responds to vertebrate dispersers, often in the form of correlated suites of morphological traits representing syndromes generally associated with attractiveness to either birds or mammals (Janson 1983; Lomascolo *et al.* 2010). There is thus a potential for three-way interactions among plants, consumers and dispersers, shaping fruit size.

Species with very large seeds may be adapted primarily to dispersal by large animals rather than to protection against small predators (Grubb *et al.* 1998), their nutrient resources being sufficient for successful germination even if they are partly consumed. However, large seeds also represent an extraordinarily attractive target for herbivores, unless they also contain high amounts of crude fibres (Ramirez & Traveset 2010) or toxic compounds (Janzen *et al.* 1977; Nahrstedt 1985) that protect them from insects (Ramirez & Traveset 2010). Small fruit and seed size may, on the other hand, act as a limiting factor for insect frugivores, requiring a certain minimum amount of resources for development (Center & Johnson 1974; Szentesi & Jermy 1995). For instance, many bruchid species are unable to colonize very flat seeds, in which the smallest dimension is smaller than the size of adult beetles (Center & Johnson 1974; Szentesi & Jermy 1995). However, many plant species with seeds of suitable size and shape are not infested by bruchids, suggesting that other factors, such as plant chemical defences, may be also important (Szentesi & Jermy 1995).

We expected that frugivorous species aim to avoid interspecific competition; therefore, we assumed that the number of species, families and orders attacking individual plant species will be overdispersed, rather than following Poisson distribution among plant species. However, multiple frugivore species avoid competition between larvae developing inside of the same fruit by (i) spatial separation, with different species limited to, for example, either pulp or seed (Janzen 1983); (ii) temporal separation, specializing on either pre-dispersal or post-dispersal phase (Pereira *et al.* 2014), or even within the same phase of ripening (Janzen 1983); or (iii) the ability to share abundant resources (pulp in particular) without competition (Wilson *et al.* 2012).

We expected that fruit size will have a significant effect on composition of frugivorous communities. This question has been rarely studied, and a single study by Ramirez and Traveset (2010) focused on fruit traits showed significant differences in traits of fruits preferred by various beetle families and by Lepidoptera. Previously, we found (on a subset of the dataset presented here) that fruit morphology influences whether a fruit will be attacked by specialist or generalist weevils or

whether the fruit will remain unattacked (Ctvrtecka *et al.* 2014). Other existing studies focus either on insects feeding on a particular plant family (Nakagawa *et al.* 2003; Hosaka *et al.* 2009) or on a particular insect taxon (Janzen 1980; Pinzon-Navarro *et al.* 2010; Grimbacher *et al.* 2013). Our current study on the other hand offers quantitative data on all three principal frugivorous insect orders (Coleoptera, Diptera and Lepidoptera) across a broad phylogenetically diverse selection of plants from the entire local plant community in a lowland rainforest. The aims of the study are (i) to quantify the abundance and taxonomic composition of frugivorous insects on individual plant species and (ii) to analyse which plant species escape attack by frugivores altogether.

METHODS

Study areas

The study was conducted from March 2008 to April 2009 in two areas approximately 100 km apart: (i) near the villages of Baitabag, Mis and Ohu within a 20 × 10-km area comprising a successional mosaic of disturbed and mature lowland rainforest (5°08'–14'S, 145°7'–41'E, 50–200 m a. s.l., Madang Province, Papua New Guinea) and (ii) in a relatively less disturbed forest near Wanang village (5°14'S, 145°11'E, 100 m a.s.l.). Vegetation in these areas is similar in species composition and has been classified as mixed evergreen rainforest on Latosol with a humid climate and mean annual temperature of 26 °C (described in detail by Ctvrtecka *et al.* 2014).

Study design

Fruits were sampled by searching a 200 to 400-ha matrix of mature and early successional forest at each site and by collecting all plant species encountered in fruiting condition. Sampling effort amounted to 1284 person-days of field work (312 person-days per site in Baitabag, Mis and Ohu and 348 person-days in Wanang). Mature or nearly mature fruits were collected from branches and the forest floor whereas decomposing fruits on the ground were avoided. A collection of fruits from an individual tree, shrub or liana on a particular day represented a single sample unit for analysis. Individual samples comprised from 1 to 1500 individual fruits and weighed between 22 and 8311 g. We employed a functional definition of individual fruit for the purpose of measurement to encompass aggregate fruits arising from the fusion of adjacent carpels (e.g. *Artocarpus* and *Ficus*). For a subset of plant species, basal area in a 50-ha forest dynamics plot at Wanang, where all tree individuals with d.b.h. > 1 cm were measured and identified (G. Weiblen, unpublished data), was used as a proxy for local abundance. We were able to calculate basal area for 218 species that were present in the plot out a total of 531 plant species from which fruits were sampled.

To investigate plant traits and fruit suitability for insect development, we measured weight and volume of the whole fruit, combined volume of seeds per fruit (in the case of many-seeded fruits), volume of mesocarp per fruit and fleshiness (% of fruit volume represented by mesocarp). One or several ripe fruits from each sample were cut along both axes and photographed. Cross-sectional area of the fruit and the seed were estimated for 268 species from diameter measurements of the photographs using Adobe Photoshop, and the volume of each was calculated as an ellipsoid ($4/3 \times 3.14 \times A/2 \times B/2 \times C/2$, where A, B and C are the length, width and height of the fruit, respectively).

We collected all reared insects (rearing procedure described in detail by Ctvrtecka *et al.* 2014), and quantitative data (i.e. abundances) on Coleoptera, Lepidoptera and Diptera (except Drosophilidae) were used in the current study. Drosophilidae were excluded because they are mostly scavengers. For qualitative analyses, all specimens (except Anthribidae (Coleoptera) and Tephritidae (Diptera)) were assigned to morphospecies. Tephritidae were not morphotyped because in our high-throughput experimental design, it was not feasible to provide emerging adults with opportunity to mature and express characteristics required for species identification. Further, we were not able to morphotype Anthribidae as they included many cryptic species. Cytochrome C oxidase subunit I (COI) sequences (Wilson 2012; Miller *et al.* 2013; Miller 2015) of morphospecies were analysed at the University of Guelph (www.boldsystems.org) to verify our species identifications. We sampled 540 Lepidoptera (490 successful sequences), 614 Coleoptera (426 sequences) and 190 Diptera (168 sequences), representing 239 species. Data released on GenBank (accession numbers GU695412–5, GU695431–2, GU695434–66, GU695468–9, GU695504–46, GU695548–58, GU695561, GU695575–80, GU695623–36, GU695639–701, GU695716–7, GU695720–1, GU695745, HM376367–75, HM376381–4, HM422448–56, HM902704–15, HQ947496–7, HQ956600–1 and HQ956613–4) include the standard fields for BARCODE data, while more data including images and host plants are available on BOLD (www.boldsystems.org; Ratnasingham and Hebert 2007, 2013), accessible using a DOI ([dx.doi.org/10.5883/DS-PNGFRUIT](https://doi.org/10.5883/DS-PNGFRUIT)). Most confirmed morphospecies were identified to species by A. Riedel and R. Thomson (Coleoptera, Curculionidae); R. Rozkosny (Diptera: Stratiomyidae; Rozkosny 2013); S. Kubik (Diptera: Chloropidae); M. Bartak (Diptera: Neriidae); A. Whittington (Diptera: Syrphidae); S. E. Miller and collaborators at the Smithsonian Institution in Washington, DC (Lepidoptera).

Insect vouchers were deposited at the Smithsonian Institution and at the Papua New Guinea Agriculture Research Institute in Port Moresby. Fruit and plant vouchers were deposited at the Papua New Guinea Forest Research Institute in Lae and at the University of Minnesota Herbarium (J. F. Bell Museum) in St Paul. Digital photographs and voucher information associated with fruit specimens were submitted to the New Guinea Atrium digital herbarium (<http://ng.atrrium-biodiversity.org/atrium>).

Data analysis

Only plant species with a total sample weight of ≥ 1 kg and with more than 50 individual fruits were included in the analyses ($n = 326$; while the other 187 plant species had too few fruits sampled – see Ctvrtecka *et al.* 2014 for more details). These thresholds represent a compromise between maximizing the number of plant species analysed and the thorough sampling of insect assemblages from every host plant species. We did not assess seed mortality but rather calculated the density of insects per fruit and per unit mass of fruit and the proportion of infested plant species. We used variance tests (Schluter 1984) and log-linear analysis of frequency tables to assess simultaneously whether insect taxa were associated with particular plant species. We tested whether the distribution of insect orders (and families) corresponded with the Poisson distribution, which would suggest that we cannot reject the hypothesis that the likelihood of colonization of a plant species by a new insect order (or family) is independent of the number of orders already present on that plant. Standard statistical tests were implemented in R.

RESULTS

In total, we analysed insects from 3088 samples weighing 2758.8 kg from 326 woody plant species representing 58 families that were sufficiently sampled by at least 50 fruits and weighing at least 1 kg in aggregate per plant species. Details on sample sizes, total number and list and ecological importance of all 531 surveyed plant species is described by Ctvrtecka *et al.* (2014).

We reared 26 130 individual insects representing three orders (Coleoptera – Ctvrtecka *et al.* 2014; Diptera – Table S1; and Lepidoptera – Sam *et al.*, in prep.) from 263 plant species, that is, 80.7% of all sufficiently sampled species (Table S1). We identified 64 species and 12 315 individuals of Coleoptera, more than 58 species and 12 174 individuals of Diptera and 141 species and 1641 individuals of Lepidoptera. Note that only a smaller subset of Tephritidae (Diptera) was identified into species (28 species were barcoded and 30 morphotyped), and we expect that the observed 8776 individuals may comprise 30–100 species in total.

The proportion of plant species infested by insects did not show a clear trend with the total weight of the fruit sample and particularly with the number of fruits collected per species, although the most massive and most numerous sample categories exhibited the highest proportion of attacked plant species (Fig. 1). Overall, the fruits of 63 out of 326 plant species (i.e. 19.3%) were not attacked by insects. The attacked species (i.e. 263/326) included 33.4% of all plant species that were attacked by a single insect order, 30% attacked by two orders and 17.2% that were attacked by all three (Coleoptera, Lepidoptera and Diptera; Fig. 2). The

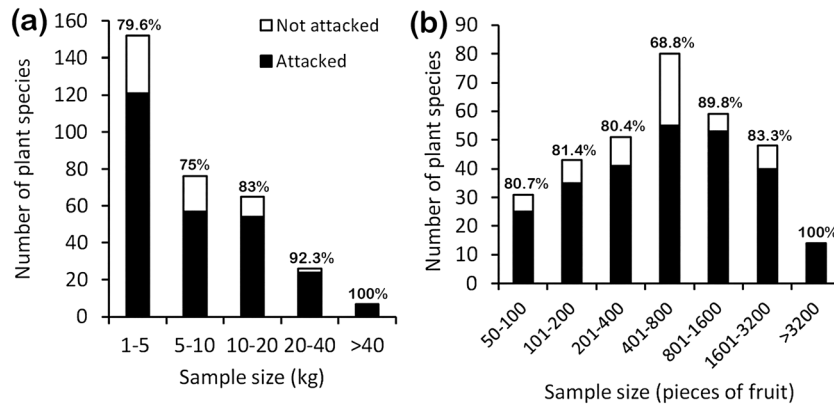


Fig. 1. The number of plant species attacked and not attacked by frugivorous insects (Coleoptera, Diptera and Lepidoptera) in categories of fruit sample weight (a) and the number of fruits per sample (b) in Papua New Guinea. Percentages indicate the proportion of plant species in each category that were attacked by frugivorous insects.

proportion of plants that escaped insect attack entirely, as well as the proportion of plant species attacked by all three orders, were higher than expected if the attacks by individual insect orders were mutually independent events (Fig. 2). A variance test (Schluter 1984) confirmed significant and positive association between insect orders ($P < 0.001$, variance ratio 1.43, $w = 467$, d.f. = 326), and the positive association between Diptera and Coleoptera and Diptera and Lepidoptera was selected as the best model in comparison with other possible combinations.

On a taxonomically finer scale, most plant species were attacked by a limited number of insect families with 80.7% (i.e. 263/326) of species hosting one to five insect families from a total of 25 frugivorous families recorded (Fig. 3). The distribution of frugivore family and order numbers per plant species was not significantly different from a Poisson expectation.

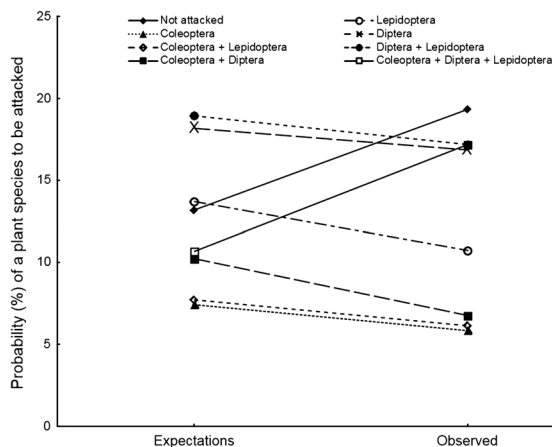


Fig. 2. Expected and observed probability of a plant species to have fruits attacked by various combinations of insect orders, on the assumption that insect orders attack plant species independently from each other.

Fruit weight, fruit volume, mesocarp volume, seed volume and fleshiness did not have significant effects on the probability that a fruit would be attacked by insects (fruit weight: $F_{1, 324} = 0.08$, $P = 0.77$; fruit volume: $F_{1, 324} = 0.06$, $P = 0.79$; seed volume: $F_{1, 324} < 0.01$, $P = 0.98$; mesocarp volume: $F_{1, 324} = 0.11$, $P = 0.74$; fleshiness: $F_{1, 324} = 0.03$, $P = 0.86$; Fig. 4). However, plant species with attacked fruits tended to have more massive (18.2 ± 2.3 vs. 16.8 ± 2.3 g), and larger (229.7 ± 47.1 vs. 203.8 ± 41.9 cm³), fruit and mesocarp (166.1 ± 37.5 vs. 140.3 ± 29.7 cm³) volume, while having similar seeds (63.7 ± 11.1 vs. 63.2 ± 16.7 cm³).

Fruits attacked by Diptera were significantly larger (KW- $H_{(2, 469)} = 10.8$, $P = 0.004$; mean \pm SE = 281.2 ± 57.8 cm³) than fruits attacked by Coleoptera and Lepidoptera (139.4 ± 17 and 169.9 ± 30.3 cm³, respectively). Similarly, fruits attacked by Diptera had larger seeds (KW- $H_{(2, 469)} = 10.91$, $P = 0.004$; mean \pm SE = 79.7 ± 13.4 cm³) than fruits attacked by Coleoptera and Lepidoptera (41.4 ± 15.2 and 46.1 ± 12.6 cm³, respectively) and had a larger volume of mesocarp (KW- $H_{(2, 469)} = 9.88$, $P = 0.007$; Diptera: 202.2 ± 46.2 cm³; Lepidoptera: 123.8 ± 37.6 cm³; Coleoptera: 97.5 ± 45.3 cm³; Fig. 5).

Individual insect families followed a similar trend, with all Diptera and the Coleoptera family Anthribidae showing preferences towards larger (Fig. 6) and heavier fruits with larger seed and mesocarp volume than Lepidoptera families. All traits were closely correlated (fruit size and fruit weight: $r = 0.90$, $P < 0.001$; fruit volume and mesocarp volume: $r = 0.98$, $P < 0.001$; fruit volume and seed volume: $r = 0.87$, $P < 0.001$). Within Coleoptera, Anthribidae showed preference for larger and heavier fruits with larger seed than Curculionidae, while Curculionidae preferred fruits with larger volume of mesocarp and higher fleshiness than Anthribidae. Individual Lepidoptera families did not differ in their preferences for fruit size and

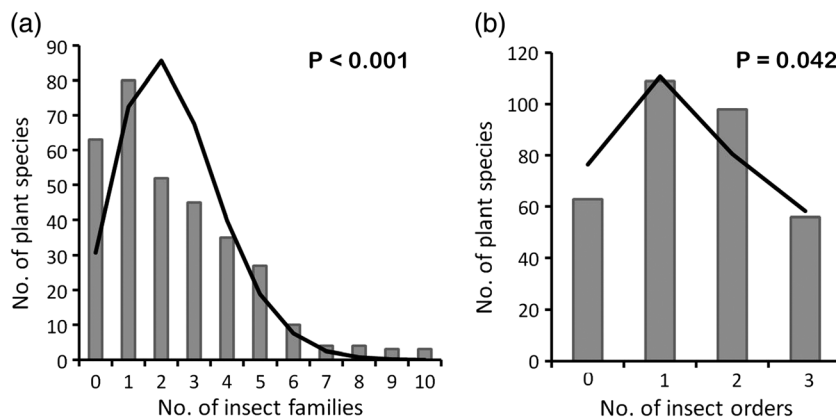


Fig. 3. Distribution of the number of insect families (a) and insect orders (b) attacking individual plant species, with expected values for a Poisson distribution (line). The observed distribution was significantly different from Poisson distribution for the number of insect families (χ^2 tests, P value is reported).

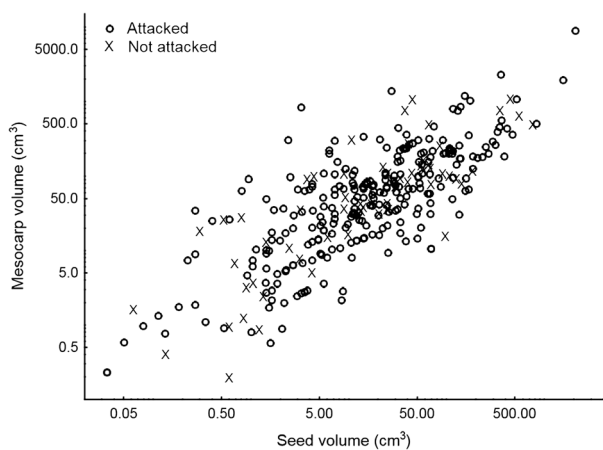


Fig. 4. Correlation ($r=0.79$, $P<0.001$) between seed and mesocarp volume for 327 plant species where fruits were or were not attacked by frugivorous insects in Papua New Guinea. Neither mesocarp volume ($F_{1, 324} = 0.35$, $P=0.24$) nor seed volume ($F_{1, 324} = 1.19$, $P=0.41$) had a significant effect on the probability of infestation.

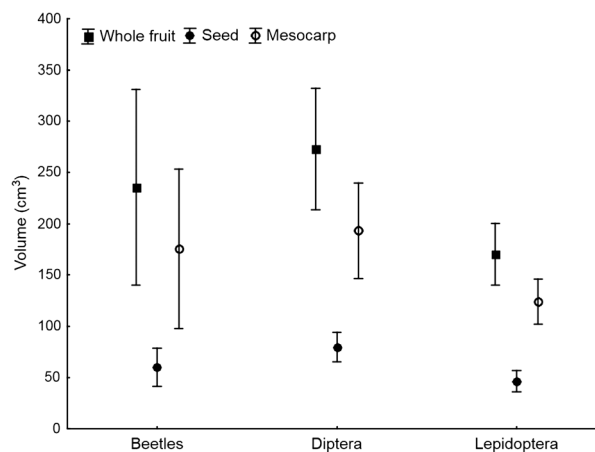


Fig. 5. Mean volume comprised by whole fruit, mesocarp or seed in fruits in Papua New Guinea attacked by frugivorous insects from different orders. Means (\pm SE) are shown for plant species attacked by Coleoptera ($n=132$), Diptera ($n=343$) and Lepidoptera ($n=296$). Note that values for plant species with fruits attacked by multiple insect orders appear multiple times in the dataset.

weight but had various preferences for fruit fleshiness (Fig. 6).

There were no significant differences among major plant lineages in the proportion of attacked species. In particular, eudicots, magnoliids and monocots suffered similar rates of attack, as was the case for core eudicots, rosids and asterids within eudicots (Fig. 7). Plant species with fruits attacked by insects were not significantly more abundant in a 50-ha forest plot (Vincent *et al.* 2014) than plants that escaped attack (ANOVA; $F_{(1, 210)} = 1.38$, $P=0.241$, mean \pm SE number of stems per attacked plant species = 755.6 ± 86.2 and per unattacked plant species = 520.1 ± 1269.6). However, plant species attacked by insects

representing two or three insect orders were significantly more abundant ($F_{(2, 169)} = 3.39$, $P=0.048$) than plant species attacked by insects representing a single order only. There was no significant relationship between density of insects (examined on per-species basis) reared from a plant species and abundance of the plant species in the forest plot.

Most of the 326 plant species exhibited low densities of frugivorous insects (Fig. 8). Each average kilogram of fruits was attacked by 10.7 ± 2.36 (mean \pm SE) insects, including 3.57 ± 0.93 Coleoptera, 6.27 ± 2.19 Diptera and 0.87 ± 0.18 Lepidoptera. Thus, we reared on average one insect from 10 fruits (average size of a collected fruit = 17.4 ± 1.9 g or 224.7 ± 38.8 cm³),

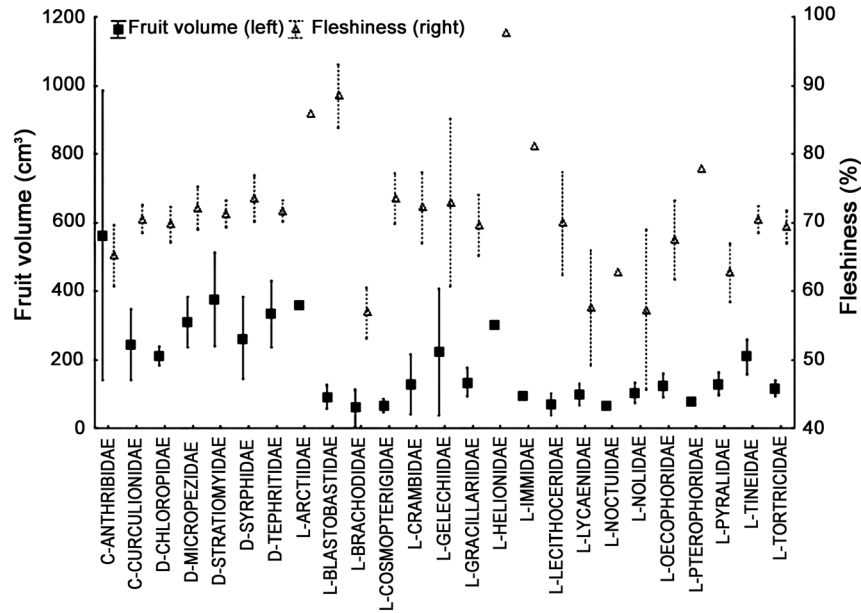


Fig. 6. Mean fruit volume and fleshiness (percentage of total volume comprised by mesocarp) in fruits in Papua New Guinea attacked by insects. Means (\pm SE) are shown for plant species attacked by different insect families. Letters in front of family names denote insect order: C, Coleoptera; D, Diptera; L, Lepidoptera.

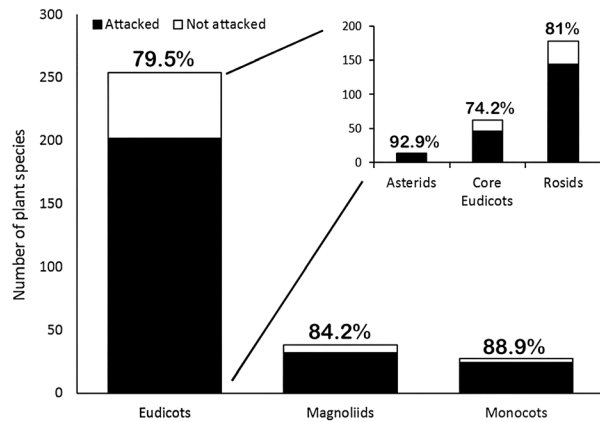


Fig. 7. Attack rate by frugivores on plant species within individual plant taxa. Main figure: $\chi^2 = 1.73$, $P = 0.42$, inserted figure: $\chi^2 = 0.88$, $P = 0.64$. The percentage of species attacked by frugivore is given for each column.

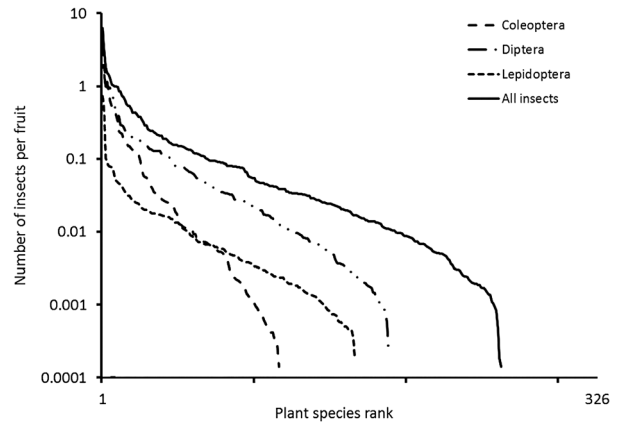


Fig. 8. Density of all frugivorous insects, and individual insect orders, in a Papua New Guinean lowland rainforest. Plant species are ranked from highest to lowest insect density for 326 plant species with samples of >1 kg and greater than 50 fruits.

including one Diptera from 14 fruits, one Coleoptera from 22 fruits and one Lepidoptera from 100 fruits. Only 72 of 326 plant species in total hosted more than one insect per 10 individual fruits, and only seven species supported densities of greater than one insect per fruit (*Leucaena leucocephala*, *Maniltoa schefferi*, *Pandanus kaerbachii*, *Xanthophyllum papuanum*, *Planchonia papuana*, *Kingiodendron alternifolium* and *Kingiodendron novoguineense*; Fig. 8). Observed median number of fruits per insect was $29.5 \pm SE 36.5$. At the other extreme, 40.1% of plant species supported densities of less than 1 insect per 100 fruits.

DISCUSSION

Approximately half of all sampled (326 out of 531) and 80% of the well-sampled (i.e. 236) plant species were attacked by insect frugivores. This is a very similar proportion as in two Venezuelan data sets, with 42.9–47.6% of plant species attacked (Raimundez 2000 – reporting results regardless of sampling effort; Ramirez & Traveset 2010 – reporting results with standardized sampling effort 100 fruits per plant species). Results from Kenya suggest a somewhat greater proportion of attacked species (910 species represented by non-standardized

samples, 57.5% attacked; Copeland *et al.* 2009). We would like to point out that sampling effort can be important in similar studies, as we could conclude that about half of the sampled trees support frugivores, while a majority (80%) of well-sampled plants support frugivores. Our findings that a large number of plants support very low densities of frugivores also lend support for not including poorly sampled species in these analyses. Other differences between current and previously published results may be caused by habitats where fruits were sampled. Results from Venezuela were based on rearings from five different habitats (forest, gallery forest, forest-savanna transition, savanna and secondary growth) in a 250-ha study area, representing a more heterogeneous and yet local data set. In Kenya, fruits were sampled from forests and coastal vegetation in multiple geographic regions from 1 to 3077 m a.s.l., so that the results represent a regional species pool rather than a community (Brown *et al.* 2014). The dataset from Kenya included all Diptera. Drosophilidae, as hyper abundant as individuals and very abundant in species of flies in decaying fruit, were included neither in the current dataset nor in aforementioned studies. Thus, we believe that sampling effort rather than exclusion of Drosophilidae from our dataset is responsible for the observed variance. Diversity of Drosophilidae in New Guinea is huge (probably 700 species; Carson & Okada 1982), and the hosts of very few of those species are known, but based on studies from tropical north Queensland in Australia, we expect that Drosophilidae may attack about 51% of fruits, and that a common species may be reared from more than one (93% species) or even more than 10 plant families (Van Klinken & Walter 2001).

Contrary to predictions (Leishman *et al.* 2000), the morphological fruit traits that we examined did not appear to explain differential fruit predation by insects. This finding agrees with the few comparable studies on the community level (Ramirez & Traveset 2010) where the incidence of attack by pre-dispersal predators was also generally independent of fruit and seed dimensions. Similarly, Beckman and Muller-Landau (2011) did not find a relationship between fruit size and seed survival due to insect predation.

We observed that heavier and larger fruits with larger seeds tended to be attacked more often in our sample. Similarly, some pre-dispersal bruchid seed predators were shown to preferentially oviposit on larger seeds (Moegenburg 1996), and larger seeds with greater energy reserves were predicted to be positively correlated with seed predation because of higher susceptibility to greater variety of large and small weevil species in acorns (Espelta *et al.* 2009). In contrast, a negative relationship between seed mass and pre-dispersal predation was found for five species of *Piper* by Greig (1993) or for Leguminosae species whose smaller seeds were attacked more by bruchids than larger seeds (Janzen 1969). All aforementioned studies are hardly comparable as they

focused on whole fruit (e.g. Greig 1993) or seeds (e.g. Ramirez & Traveset 2010) of various sizes and on various predators. For example, some studies focused on relatively small-seeded plants (Ramirez & Traveset 2010 – avg. fruit = 4.15 g; Greig 1993 – fruit = 0.05–6 mg) in comparison with the large-seeded flora (avg. fruit = 18 g) sampled by us of New Guinea (see also discussion by Mack 1993).

Fruits attacked by Diptera were significantly larger than fruits attacked by Coleoptera and Lepidoptera. The host range of most Diptera species (except Tephritidae, which were not identified to species, and Drosophilidae, which were not sampled) was also extremely wide across plant lineages. We suggest that this pattern reflects the extreme polyphagy of many Diptera species, where numerous large fruit species were included in the diet of Diptera (Ctvrtecka *et al.* 2014). Similar polyphagy, where larvae are predominantly pulp feeders and develop in ripening fruits with mature seeds, has been reported for particular tephritid species (Webber & Woodrow 2004; Wilson *et al.* 2012). This feeding mode appears to have little impact on seed survival, but may affect fruit dispersers either positively or negatively (Clarke *et al.* 2005; Wilson *et al.* 2012). In contrast, coleopteran seed predators typically avoided large-fruited species usually containing seeds protected by strong and massive endocarp (Ctvrtecka *et al.* 2014).

The most frequently attacked plant species, with a density of greater than one insect per fruit, include three species from the family Fabaceae (*M. schefferi*, *K. alternifolium* and *K. novoguineense*) with large seeds and thin non-fleshy mesocarp. These tree species were associated with four to five internal seed-eating weevil species and with a different dominant seed predator in each case. The fourth most attacked plant species, *L. leucocephala*, was also a legume. This species, with about 15–20 seeds per fruit, was attacked by external seed-eating anthribids. Further two species, *Pouteria maclayana* and *X. papuanum*, both with big-sized fruits and massive fleshy mesocarp, and *P. kaembachii*, with fibrous fleshy mesocarp and small seeds, were attacked by flies from three different families, but particularly stratiomyids. The last most attacked *P. papuana* with middle-sized fruits and relatively thin mesocarp was especially attacked by one smaller-sized Dipteran species from family Chloropidae.

In summary, gross fruit size, when characterized simply by volume, mass and fleshiness, appears to have no predictable effect on the composition of frugivorous insect communities. Fruit chemistry affecting palatability and anatomical differences affecting the mechanical properties of fruits add complexity to the picture. Our results suggest that there may be universally unpalatable plant species as well as others that are exceptionally suitable for all three insect orders. The combined assessment of attack on fruits and seeds by all three principal insect

orders confirmed the conclusion based on the study of individual orders (Ctvrtecka *et al.* 2014) that the infestation rates of fruits are generally too low for frugivores in New Guinea lowland rainforest to act as important density-dependent mortality factors mediating plant species coexistence as implied by the Janzen–Connell hypothesis (Janzen 1970; Connell 1971). It may be possible that the generally large-seeded flora of Papua New Guinea (PNG) (Marck 2000; or at least its part studied here) is primarily adapted to dispersers (i.e. to birds with preferences for large fruits) rather than to frugivorous insect. The second important assumption of density-dependent effect, for example, high rate of specialized seed predators, was also weak. The portion of specialists including monophagous species and species feeding on congeneric host plants decreased from 65% of weevil species (Coleoptera; Ctvrtecka *et al.* 2014) to 26% of Lepidoptera (Sam *et al.*, in prep.) and to no specialized Diptera (i.e. subset of Diptera analysed and identified into species in current study). We suggest that this finding mirrors food preferences of particular insect frugivorous orders from prevailing seed eaters (Coleoptera) to complete pulp eater species (Diptera).

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REFERENCES

- Adamski D., Copeland S. R., Miller E. S., Hebert N. D. P., Darrow K. & Luke Q. (2010) A review of African Blastobasinae (Lepidoptera: Gelechioidea: Coleophoridae), with new taxa reared from native fruits in Kenya. *Smithsonian Contrib. Zool.* **630**, 1–68.
- Borcherding R., Paarmann W., Nyawa S. B. & Bolte H. (2000) How to be a fig beetle? observations of ground beetles (*Col., Carabidae*) associated with fruitfalls in a rain forest of Borneo. *Ecotropica* **6**, 169–80.
- Beckman N. G. & Muller-Landau H. C. (2011) Linking fruit traits to variation in predispersal vertebrate seed predation, insect seed predation, and pathogen attack. *Ecology* **92**, 2131–40.
- Brown J. W., Copeland D. S. R., Aarvik L., Miller E. S., Rosati M. E. & Luke Q. (2014) Host records for fruit-feeding Afrotropical Tortricidae (Lepidoptera). *African Entomol.* **22**, 343–76.
- Carson H. L. & Okada T. (1982) Drosophilidae of New Guinea. In: *Biogeography and Ecology of New Guinea* pp. 675–87. W. Junk, The Hague.
- Center D. T. & Johnson D. C. (1974) Coevolution of some seed beetles (Coleoptera: Bruchidae) and their hosts. *Ecology* **55**, 1096–103.
- Christensen C. M. (1972) Microflora and seed deterioration. In: *Viability of Seeds* pp. 59–93. Chapman & Hall, London.
- Clarke A. R., Armstrong K. F., Carmichael A. E. *et al.* (2005) Invasive phytophagous pests arising through a recent tropical evolutionary radiation: the *Bactrocera dorsalis* complex of fruit flies. *Annu. Rev. Entomol.* **50**, 293–19.
- Connell J. H. (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: *Dynamics of Populations* pp. 298–312. Pudoc, Wageningen.
- Copeland S. R., Wharton A. R., Luke Q. *et al.* (2006) Geographic distribution, host fruit, and parasitoids of African fruit fly pests *Ceratitidis anonae*, *Ceratitidis cosyra*, *Ceratitidis fasciventris*, and *Ceratitidis rosa* (Diptera: Tephritidae) in Kenya. *Ann. Entomol. Soc. Am.* **99**, 261–78.
- Copeland S. R., Luke Q. & Wharton A. R. (2009) Insect reared from the wild fruits of Kenya. *J. East African Nat. Hist.* **98**, 11–66.
- Ctvrtecka R., Sam K., Brus E., Weiblen G. D. & Novotny V. (2014) Frugivorous weevils are too rare to cause Janzen–Connell effects in New Guinea lowland rainforest. *J. Trop. Ecol.* **30**, 521–35.
- Delobel B. & Delobel A. (2006) Dietary specialization in European species groups of seed beetles (Coleoptera: Bruchidae: Bruchinae). *Oecologia* **149**, 428–43.
- Espelta J. M., Bonal R. & Sanchez-Humanes B. (2009) Predispersal acorn predation in mixed oak forests: interspecific differences are driven by the interplay among seed phenology, seed size and predator size. *J. Ecol.* **97**, 1416–23.
- Greig N. (1993) Predispersal seed predation on five *Piper* species in tropical rainforest. *Oecologia* **93**, 412–20.
- Grimbacher P. S., Nichols C., Wardhaugh C. W. & Stork N. E. (2013) Low host specificity of beetles associated with fruit falls in lowland tropical rainforest of north-east Australia. *Aust. J. Entomol.* **53**, 75–82.

- Grubb P. J., Metcalfe D. J., Grubb E. A. A. & Jones G. D. (1998) Nitrogen-richness and protection of seeds in Australian tropical rainforest: a test of plant defence theory. *Oikos* **82**, 467–82.
- Herrera C. M. (1982) Defense of ripe fruits from pests: its significance in relation to plant–disperser interactions. *Am. Naturalist* **120**, 218–41.
- Herrera C. M. (1987) Flower and fruit biology in southern Spanish Mediterranean shrublands. *Ann. Mo. Bot. Gard.* **74**, 69–78.
- Hoddle S. M. & Hoddle D. C. (2008) Lepidoptera and associated parasitoids attacking hass and non-hass avocados in Guatemala. *J. Econ. Entomol.* **101**, 1310–16.
- Hosaka T., Yumoto T., Kojima H., Komai F. & Noor N. S. M. (2009) Community structure of pre-dispersal seed predatory insects on eleven *Shorea* (Dipterocarpaceae) species. *J. Trop. Ecol.* **25**, 625–36.
- Janson C. H. (1983) Adaptation of fruit morphology to dispersal agents in a neotropical forest. *Science* **219**, 187–9.
- Janzen D. H. (1969) Seed-eaters versus seed size, number, toxicity and dispersal. *Evolution* **23**, 1–27.
- Janzen D. H. (1970) Herbivores and the number of tree species in tropical forests. *Am. Naturalist* **104**, 501–28.
- Janzen D. H. (1971) Seed predators by animals. *Annu. Rev. Ecol. Syst.* **2**, 465–92.
- Janzen D. H., Juster H. B. & Belle A. (1977) Toxicity of secondary compounds of the seed-eating larvae of the Bruchid beetle *Callosobruchus maculatus*. *Phytochemistry* **16**, 223–7.
- Janzen D. H. (1979) How to be a fig. *Annu. Rev. Ecol. Syst.* **10**, 13–51.
- Janzen D. H. (1980) Specificity of seed-attacking beetles in a Costa Rican deciduous forest. *J. Ecol.* **68**, 929–52.
- Janzen D. H. (1983) Larval biology of *Ectomyelois muriscis*, (Pyralidae: Phycitinae), a Costa Rican fruit parasite of *Hymenaea courbaril* (Leguminosae: Caesalpinioideae). *Brenesia* **21**, 387–93.
- Kergoat G. J., Delobel A., Fediere G. & Silvain J. F. (2005) Both host-plant phylogeny and chemistry have shaped the African seed-beetle radiation. *Mol. Phylogenet. Evol.* **35**, 602–11.
- Lyal C. H. C. & Curran L. M. (2000) Seed-feeding beetles of the weevil tribe Mecesolobini (Insecta: Coleoptera: Curculionidae) developing in seeds of trees in the Dipterocarpaceae. *J. Nat. Hist.* **34**, 1743–847.
- Lyal C. H. C. & Curran L. M. (2003) More than black and white: new genus of nanophyinae seed predators of Dipterocarpaceae and a review of *Meregallia* Alonso-Zarazaga (Coleoptera: Curculionidae). *J. Nat. Hist.* **37**, 57–105.
- Lomascolo S. B., Levey D. J., Kimball R. T., Bolker B. M. & Alborn H. T. (2010) Dispersers shape fruit diversity in *Ficus* (Moraceae). *Proc. Natl. Acad. Sci. U. S. A.* **107**, 14668–72.
- Leishman M. R., Wright I. J., Moles A. T. & Westoby M. (2000) The evolutionary ecology of seed size. In: *Seeds: The Ecology of Regeneration in Plant Communities* pp. 31–57. CAB International, Wallingford.
- Mack A. L. (1993) The sizes of vertebrate-dispersed fruits: a neotropical-paleotropical comparison. *Am. Nat.* **142**, 840–56.
- Mack A. L. (2000) Did fleshy fruit pulp evolve as a defence against seed loss rather than as a dispersal mechanism? *J. Biosci.* **25**, 93–97.
- McQuate T. G., Follett A. P. & Yoshimoto M. J. (2000) Field infestation of rambutan fruits by internal-feeding pests in Hawaii. *J. Econ. Entomol.* **93**, 846–51.
- Miller S. E., Hrcek J., Novotny V., Weiblen G. D. & Hebert P. D. N. (2013) DNA barcoding of caterpillars (Lepidoptera) from Papua New Guinea. *Proc. Entomol. Soc. Wash.* **115**, 107–9.
- Miller S. E. (2015) DNA barcoding in floral and faunal research. In: *Descriptive Taxonomy: The Foundation of Biodiversity Research* pp. 296–311. Cambridge University Press, Cambridge.
- Mitsui H., Beppu K. & Kimura M. T. (2010) Seasonal life cycle and resource uses of flower- and fruit-feeding drosophilid flies (Diptera: Drosophilidae) in central Japan. *Entomol. Sci.* **13**, 60–7.
- Moegenburg M. S. (1996) Sabal palmetto seed size: sources of variation, choices of predators, and consequences for seedlings. *Oecologia* **106**, 539–43.
- Nahrstedt A. (1985) Cyanogenic compounds as protecting agents for organisms. *Plant Sys. Evol.* **150**, 35–47.
- Moore D. (2001) Insects of palm flowers and fruits. In: *Insects on Palms* pp. 233–66. CAB International, Wallingford, UK.
- Nakagawa M., Itoioka T., Momose K. et al. (2003) Resource use of insect seed predators during general flowering and seeding events in a Bornean dipterocarp rainforest. *Bull. Entomol. Res.* **93**, 455–66.
- Novotny V., Clarke A. R., Drew R. A. I., Balagawi S. & Clifford B. (2005) Host specialization and species richness of fruit flies (Diptera: Tephritidae) in a New Guinea rainforest. *J. Trop. Ecol.* **21**, 61–77.
- Pinzon-Navarro S., Barrios H., Murria C., Lyal C. H. C. & Vogler A. P. (2010) DNA-based taxonomy of larval stages reveals huge unknown species diversity in neotropical seed weevils (genus *Conotrachelus*): relevance to evolutionary ecology. *Mol. Phylogenet. Evol.* **56**, 281–93.
- Pereira A. C. F., Fonseca F. S. A., Mota G. R. et al. (2014) Ecological interactions shape the dynamics of seed predation in *Acrocomia aculeata* (Arecaceae). *PLoS One* **9**: e98026.
- Raga A., Souza-Filho D., Machado R. A., Sato M. A. & Siloto R. C. (2011) Host ranges and infestation indices of fruit flies (Tephritidae) and lance flies (Lonchaeidae) in São Paulo State, Brazil. *Fla. Entomol.* **94**, 787–94.
- Raimundez, E. A. (2000) Evaluacion de algunos aspectos reproductivos y ecologicos en especies de plantas de comunidades naturales de la Gran Sabana (Parque Nacional Canaima, Estado Bolivar). Tesis Doctoral, Universidad Central de Venezuela. Post-grado Botanica, Caracas, Venezuela, 203pp, Mimeografía.
- Ramirez N. & Traveset A. (2010) Predispersal seed predation by insects in the Venezuelan central plain: overall patterns and traits that influence its biology and taxonomic groups. *Perspect. Plant Ecol. Evol. Syst.* **12**, 193–209.
- Ratnasingham S. & Hebert P. D. N. (2007) The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* **7**, 355–364. doi: 10.1111/j.1471-8286.2007.01678.x.
- Ratnasingham S. & Hebert P. D. N. (2013) A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PLoS One* **8**, e66213, DOI: 10.1371/journal.pone.0066213.
- Rozkosny R. (2013) A new species of *Glochimyia* Kertész from Papua New Guinea (Diptera: Stratiomyidae, Pachygastrinae). *Zootaxa* **3693**, 379–86.
- Schluter D. (1984) A variance test for detecting species associations, with some example applications. *Ecology* **65**, 998–1005.
- Slater J. A. (1972) Lygaeid bugs (Hemiptera: Lygaeidae) as seed predators of figs. *Biotropica* **4**, 145–51.
- Szentesi A. & Jermy T. (1995) Predispersal seed predation in leguminous species: seed morphology and bruchid distribution. *Oikos* **73**, 23–32.
- Tan M. K. (2011) The Copiphorini (Orthoptera: Tettigoniidae: Conocephalinae) in Singapore. *Nature in Singapore* **4**, 31–42.

- Van Klinken R. D. & Walter G. H. (2001) Larval hosts of Australian Drosophilidae (Diptera): a field survey in subtropical and tropical Australia. *Aust. J. Entomol.* **40**, 163–79.
- Vincent J. B., Henning B., Saulei S., Sosanika G. & Weiblen G. D. (2014) Forest carbon in lowland Papua New Guinea: local variation and the importance of small trees. *Austral Ecol.* DOI: 10.1111/aec.12187.
- Webber L. B. & Woodrow E. I. (2004) Cassowary frugivory, seed defleshing and fruit fly infestation influence the transition from seed to seedling in the rare Australian rainforest tree, *Ryparosa* sp. nov. 1 (Achariaceae). *Funct. Plant Biol.* **31**, 505–16.
- Weiblen G. D. (2002) How to be a fig wasp. *Annu. Rev. Entomol.* **47**, 299–330.
- Wilson J. J. (2012) DNA barcodes for insects. In: *DNA Barcodes: Methods and Protocols* pp. 17–46. Springer, New York.
- Wilson J. A., Schutze M., Elmouttie D. & Clarke R. A. (2012) Are insect frugivores always plant pests? the impact of fruit fly (Diptera: Tephritidae) larvae on host plant fitness. *Arth.-Plant Int.* **6**, 635–47.
- Wright S. J. (1990) Cumulative satiation of a seed predator over the fruiting season of its hosts. *Oikos* **58**, 272–76.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Table S1. Host range and abundance of reared Diptera species.

Table S2. Abundance of frugivorous Coleoptera, Lepidoptera and Diptera on individual plant species.