Sequential Florivory/Saproflorivory of *Anaxagorea crassipetala* (Annonaceae) by *Diathoneura tessellata* (Drosophilidae)

G. E. COLLIER[1] AND J. E. ARMSTRONG[2]

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ABSTRACT *Diathoneura tessellata* Duda, 1925 (Diptera: Drosophilidae) uniquely and effectively uses the fleshy tepals of *Anaxagorea crassipetala* (Annonaceae), a small, understory tree of the primary lowland rain forest of Costa Rica, as a larval substrate and pupation site. This study is the first to document 1) the brood substrate for larvae of this species and the 2) use of flowers in the Annonaceae as a drosophilid larval substrate. Oviposition into the tough, immature flower buds is made possible by an enlarged oviscape. This relationship is unique in that these flowers support two sequential cohorts of larvae, one cohort mining the living tepals of immature and mature flowers and the second cohort consuming the fallen, postanthesis tepals. We refer to this phenomenon as sequential florivory/saproflorivory. The first cohort consists of fewer, larger larvae, whereas the second cohort consists of more numerous, smaller larvae. Both cohorts exhibit a female skewed sex ratio.

KEY WORDS Annonaceae, Costa Rica, Drosophilidae, florivory, saproflorivory

Drosophilids exploit a diversity of resources as larval food sources. Most often these are decaying plant tissue (i.e., fruit, cactus rots, and tree fluxes) and fungi (DeSalle and Grimaldi 1991). However, several species have been bred out of fallen flowers and therefore are saproflorivorous. These species commonly include members of the tripunctata species group (Pipkin et al. 1966, Feinstein et al. 2007), some members of the genus *Zygothrica* (Grimaldi 1987, Pipkin et al. 1966, Sakai 2002, Santos and Vilela 2005, Feinstein et al. 2007), and a few other species [e.g., *Drosophila* (*D.*) *bromeliae* Sturtevant) (Sakai 2002). Larvae of other species do occur in open flowers and hence are florivorous. The flavopilosa species group of the genus *Drosophila* (Wheeler et al. 1962, Pipkin et al. 1966, Brncic 1983, Santos and Vilela 2005) is the best known example; however, in this case the larvae do not consume the petals, but rather feed upon pollen. The onchyophora species group (Hunter 1992, Vilela and Bachli 1990) from high elevations (>2,500 m) in the Andes are known to oviposit on flowers of several different plant genera. Members of the African genus *Lissocephala* are unusual in that larvae develop within the immature fig (*Ficus* spp.) synconium (Lachaise et al. 1982).

However, for many members of this diverse group of flies, the larval substrate is completely unknown. *Diathoneura* (Diptera: Drosophilidae) is a basal genus of the subfamily Drosophilinae (Grimaldi 1990) that currently possesses 32 recognized species (Duda 1925, Vilela and Bachli 1990, Nguyen 2003). Pipkin et al. (1966) anecdotally reported that adults of one to three Panamanian species of this genus (then called Clastoteromyia) were bred out of fallen perianths of *Heliconia vellerigera* (Heliconiaceae) and *Centropogon coccineus* (Campanulaceae). For >40 yr, Pipkin et al. (1966) has been the primary authority for the assertion that the remaining species also use flowers as a brood substrate.

Anaxagorea crassipetala (Annonaceae) is an understory tree of the primary lowland rain forest of Costa Rica. The trees are small, 1.5–8.8-cm trunk diameter at breast height and 4–8 m tall. This species has a patchy distribution determined by topography; it grows on the shoulders and slopes along ridges and water courses. Within a patch this species of tree can be very common with a mean trunk to trunk distance to its nearest conspecific neighbor of 3.2 m (range, 0.5–12.9 m). Flowering occurs during October and November, averaging 0.84 mature flowers per tree per d (range, 0.09–3.30; $N = 37$) over the 42 d this population flowered (Armstrong and Marsh 1997).

The flowers exhibit a very precise 24-h anthesis beginning at first light (Armstrong and Marsh 1997). The flowers are pendant, and the perianth consists of three whorls of three tepals each. The outer whorl of tepals is sepaloid, whereas the middle and the inner whorls are petaloid (Fig. 1). The tepals of the middle whorl are very fleshy, making up 64% of the total floral biomass. The tepals of the inner whorl are smaller and much less fleshy than those of the middle whorl. At the end of anthesis, the two inner whorls of tepals and the anthers shatter and fall to the forest floor.

While investigating the flowering of this tree, three categories of insect visitors were found (Armstrong

[1] Corresponding author: Department of Biological Science, University of Tulsa, 800 S. Tucker Ave., Tulsa, OK 74104-3189 (e-mail: glen-collier@utulsa.edu).

[2] Behavior, Ecology, Evolution, and Systematics, Campus Box 4120, School of Biological Sciences, Illinois State University, Normal, IL 6 1790-4120.

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Fig. 1. An open flower of *A. crassipetala*. The labels o.w., m.w., and i.w. refer to the sepaloid outer whorl of tepals, the fleshy middle whorl of tepals, and the small inner whorl of tepals, respectively. 2.8 × life-size. (Online figure in color.)

and Marsh 1997). Only beetles (Coleoptera: Nitidulidae, Staphylinidae) enter the closed chamber formed by the inner two whorls of tepals and are presumed the likely pollinators. A new species of *Cyrionyx* (Curculionidae: Baridinae) oviposits in young flower buds and is an ovule predator. The feeding of a single larva upon the pistils causes early bud abortion of as much as 43% of all buds (Armstrong and Marsh 1997). The third category was represented by a drosophilid fly seen on the flowers and its presumed larvae were found within the fallen, middle whorl tepals. Neither the adults nor the larvae were ever seen within the perianth, and therefore play no direct role in the pollination biology of *Anaxagorea.* Thus, the drosophilids are nonpollinating florivores. However, although numerous observations were made during two prior field seasons (1992 and 2000), the dipteran florivory was not the primary object of study until our recent (2007) collaboration.

The purpose of the 2007 fieldwork was to determine the identity of the adult drosophilid seen on the flowers of *A. crassipetala*, to verify that the larvae present within the tepals of these flowers were of the same species, and to collect basic data on the life cycle of this species.

Materials and Methods

A. crassipetala trees located within the primary forest at La Selva Biological Station, Costa Rica, were monitored during their October to November flowering season in 1993, 2000, and 2007. Specifically, two populations of trees in the primary forest were checked each morning and evening for fallen tepals, open flowers, and immature flower buds. Twenty small flower buds were marked and measured periodically from apex to base of the perianth to monitor rate of growth leading to anthesis. Five of these remained intact through anthesis. The average of these five over the preceding days was used to illustrate the rate of bud elongation. Another set of eighteen immature buds of various sizes were collected and dissected to determine the number and size of larvae present.

In 2000, as part of an experimental manipulation, 8-mm-long flower buds were bagged with netting to prevent access to the flowers by insects. The netting was removed at the beginning of anthesis, thereby allowing insects access to the flowers. During 2007, the flowers were similarly netted, but in this instance, some flowers were netted at the beginning of anthesis such

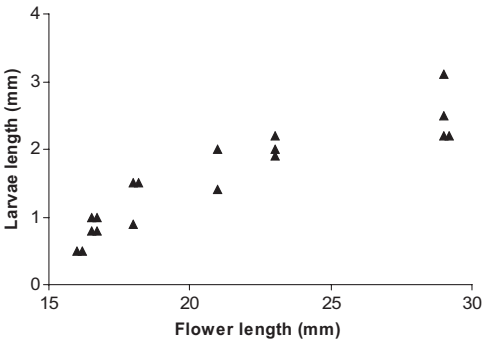

Fig. 2. Larval size versus flower bud length. Eighteen immature flower buds were dissected and the size of the flower bud and the size of the larvae found within them are plotted. The point for 16-mm buds represents two separate buds of that size that each contained a single 0.5-mm egg. No larvae or eggs were found in buds smaller than 16 mm.

that no further visitation could take place. All fleshy tepals were marked and retrieved for examination.

Fallen petalloid tepals, both the middle and inner whorls, were recovered and dissected on the first, second, and third day postanthesis to determine the number and size of larvae and pupae found. Adult insects were reared from the fleshy tepals placed on damp cotton pads in netted plastic cups.

Specimens of adults, larvae, and pupae were sexed, measured, and spirit preserved for later examination. Selected specimens were sent to Dr. David Grimaldi (American Museum of Natural History, New York, NY) for identification.

Results

The majority of fresh fallen fleshy tepals (middle whorl) contained dipteran larvae (77.5% [N = 84; 2007] to 88.0% [N = 526; 2000]). The innermost whorl of tepals never harbored insect larvae, nor did the thin, hard outermost sepaloid tepals. Armstrong and Marsh (1997) reported that middle whorl tepals 12, 36, and 60 h postanthesis had an average of 2.6, 3.2, and 12.5 larvae per tepal. Larvae found in the newly fallen fleshy tepals in 2007 were nearly 3 mm in length and were likely third-instar larvae. Indeed, tepals from intact open flowers were found to harbor larvae as well. Clearly, the majority of normal-looking fleshy tepals harbor mature larvae of this dipteran. All of the larvae seemed identical, presumably of a single species of Drosophilidae.

Similar larvae, although of decreasing size, were found in the tepals of flower buds down to 16 mm in length (Fig. 2). No larvae were found in smaller buds. The length and diameter of the larval feeding tract(s) within the tepal also decreases in earlier bud stages. Two small (≈0.5 mm) oval eggs were dissected from two separate tepals from 16 mm buds. Buds elongated such that 16-mm-long flower buds take 4–7 d to reach a length of 23–26 mm and flower (Fig. 3), a process largely involving cell enlargement resulting in fleshy tepals that are less dense at maturity.

Fleshy tepals from flowers netted at the beginning of anthesis were found to have 2.55 larvae per tepal (±0.51; N = 21). Tepals from flowers whose netting was removed at the beginning of anthesis were found to have 11.94 larvae per tepal (±12.71; N = 89). In total, mixed aged tepals with dipteran larvae averaged 12.47 larvae per tepal (N = 15; 1993), 13.51 larvae per tepal (N = 93; 2007), and 13.89 larvae per tepal (N = 182; 2000).

Dipteran pupae were observed in tepals as early as 24 h postanthesis, and these pupae were most often found at the narrow, proximal end of the tepal, with their spiracles extruded through the epidermis. Although some pupae remained within the tepal epidermis, many larvae crawled out of the decaying tepals to pupate in the moist cotton. Adults were observed to eclose 5 d after pupation.

Adult flies eclosing from collected tepals displayed a highly skewed female-biased sex ratio (1.82; P [χ^2] = 4.1×10^{-6}; N = 251) (Fig. 4A). The first adults to eclose were all males; some females eclosed on the second day, and from the third day on the ratio was female biased. The first adults of either sex to eclose, those that came from preflowering ovipositions, were larger than those of the same sex that eclosed later, which came from ovipositions made on the day of flowering. A significant female-biased size differential,

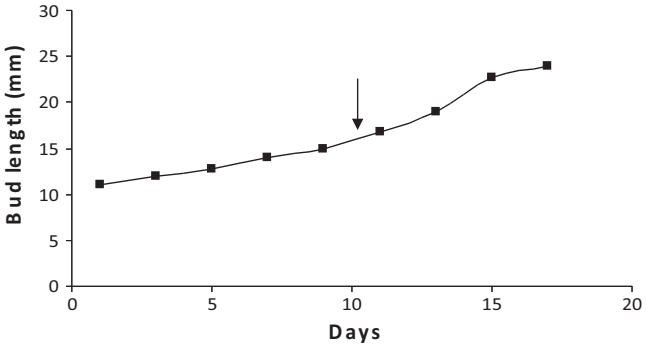

Fig. 3. Flower bud elongation rate up to anthesis. Five immature flower buds were marked and measured on successive days. The points are the average of these measurements at 2-d intervals. The line illustrates the increase in bud size over time. The arrow indicates the smallest bud size in which eggs or larvae were found.

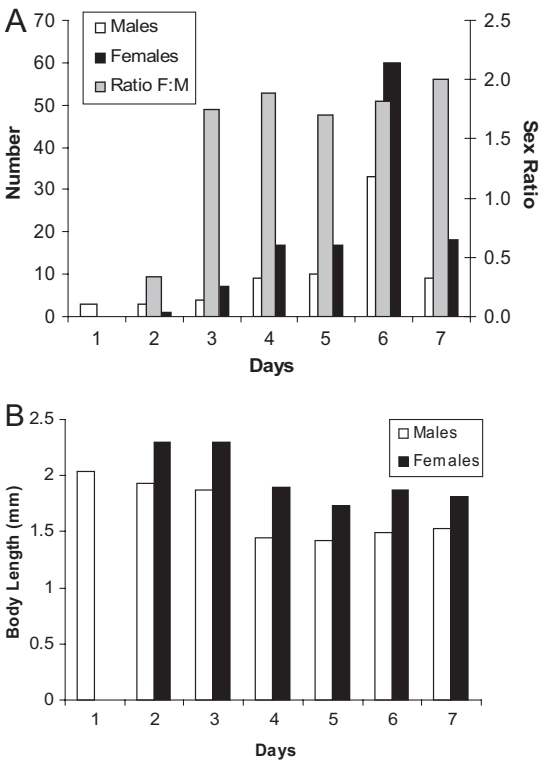

Fig. 4. Eclosion data for *D. tessellata* reared from fallen tepals of *A. crassipetala*. Tepals were placed on moist cotton in plastic cups covered with netting and kept at ambient temperature. Day 1 is the fifth day after anthesis. (A) Number of males and females, and sex ratio of *D. tessellata* by day of eclosion. (B) Mean body length of male and female flies by day of eclosion.

typical of most drosophilids, also was observed across both broods (Fig. 4B; least squares mean body lengths, males = 1.62 [±0.03 SE] mm, females = 1.94 [±0.03 SE] mm; $P < 0.0001$).

All but two of the 251 adults emerging from these tepals in 2007 were *Diathoneura tessellata* Duda, 1925 (Diptera: Drosophilidae) (D. Grimaldi, personal communication). The female is remarkable in possessing a prominent oviscape (Fig. 5) that presumably enables the female to pierce the tough epidermis and denser mesophyll of the immature perianth. Of the oviscapes of females of seven species of *Diathoneura* figured by Vilela and Bachli (1990), *D. tessellata* was by far the most extended. The two non-*Diathoneura* adults were females of an undetermined *Drosophila* species. These two specimens eclosed on next to the last day of eclosions monitored for a large set of fallen tepals (Fig. 4) and are assumed to be an opportunistic saprophagous species that oviposited on the fallen tepals before they were collected.

Discussion

The fleshy tepals of *A. crassipetala* are a very limited and ephemeral larval substrate that is used effectively (i.e., nearly all the fleshy tepals are affected) and uniquely (i.e., only one species ecloses from the tepals) by this species of *Diathoneura.* At the same time that these observations support and extend the inference that *Diathoneura* larvae are florivorous, they also illustrate an unexpected complexity of larval florivory. Although nonpollinating florivory is a little studied phenomenon (Frame 2003), a large guild of saproflorivorous insects may exist (Feinstein et al. 2007, 2008). The latter conclusion derives from monitoring adults eclosing from fallen flower petals and androecia. Examples of drosophilid larvae living in intact flowers until now was limited to those larvae that feed on pollen (Brncic 1983, Wheeler et al. 1962) or within the fig synconium (Lachaise et al. 1982). Our data clearly show that florivory and saproflorivory can occur sequentially in the same host by a common larval herbivore.

Oviposition by females of *D. tessellata* takes place in two temporally separate episodes. A few ovipositions take place in flower buds some 5–7 d before anthesis, resulting in the two to three third-instar larvae found in newly fallen tepals. These pupate during the first day after falling and eclose as adults 5 d later. Together, these data suggest an egg-to-adult developmental time of 12 to 14 d. The second episode of oviposition occurs on the day of flowering. This is responsible for a larger number of smaller larvae that begin to show up in the fleshy tepals 1 to 2 d after anthesis and that eclose later (Fig. 4A). This inference is supported by the flower exclusion data. If insects are excluded from the flowers until the day of anthesis, the first cohort of larvae are not found in the fallen, fleshy tepals. However, if insects are excluded from access to the flowers from just before anthesis until the fallen, fleshy tepals are collected, then the second cohort of larvae is not found. The second oviposition episode results in the majority (88.4%) of all adults eventually recovered from the tepals.

We call this phenomenon sequential florivory/saproflorivory to emphasize that one cohort of larvae develops in the living, fleshy tepals of the intact flower, and a second cohort develops in decaying tissue of the postanthesis fleshy tepals.

One consequence of the dual cohorts of *Diathoneura* larvae is that each cohort develops in what is potentially a very different environment. The first grows within the living tissue of flower tepals before, during, and immediately after anthesis. This cohort results in larger adults (Fig. 4B) either as a consequence of the intact tepal being a higher quality food source and/or because the number of larvae present per tepal is lower than that seen for the second cohort. The second cohort is deposited within the tepals during anthesis, when the flowers are odifereous and easy to locate, and develops in decomposing tissue lying on the forest floor. Occasionally, other insect larvae were observed within this decomposing tissue. Possible explanations for smaller sized adults include lower food quality of the decomposing tissue, resource limitation of the decomposing tissue, conspecific competition, or a combination.

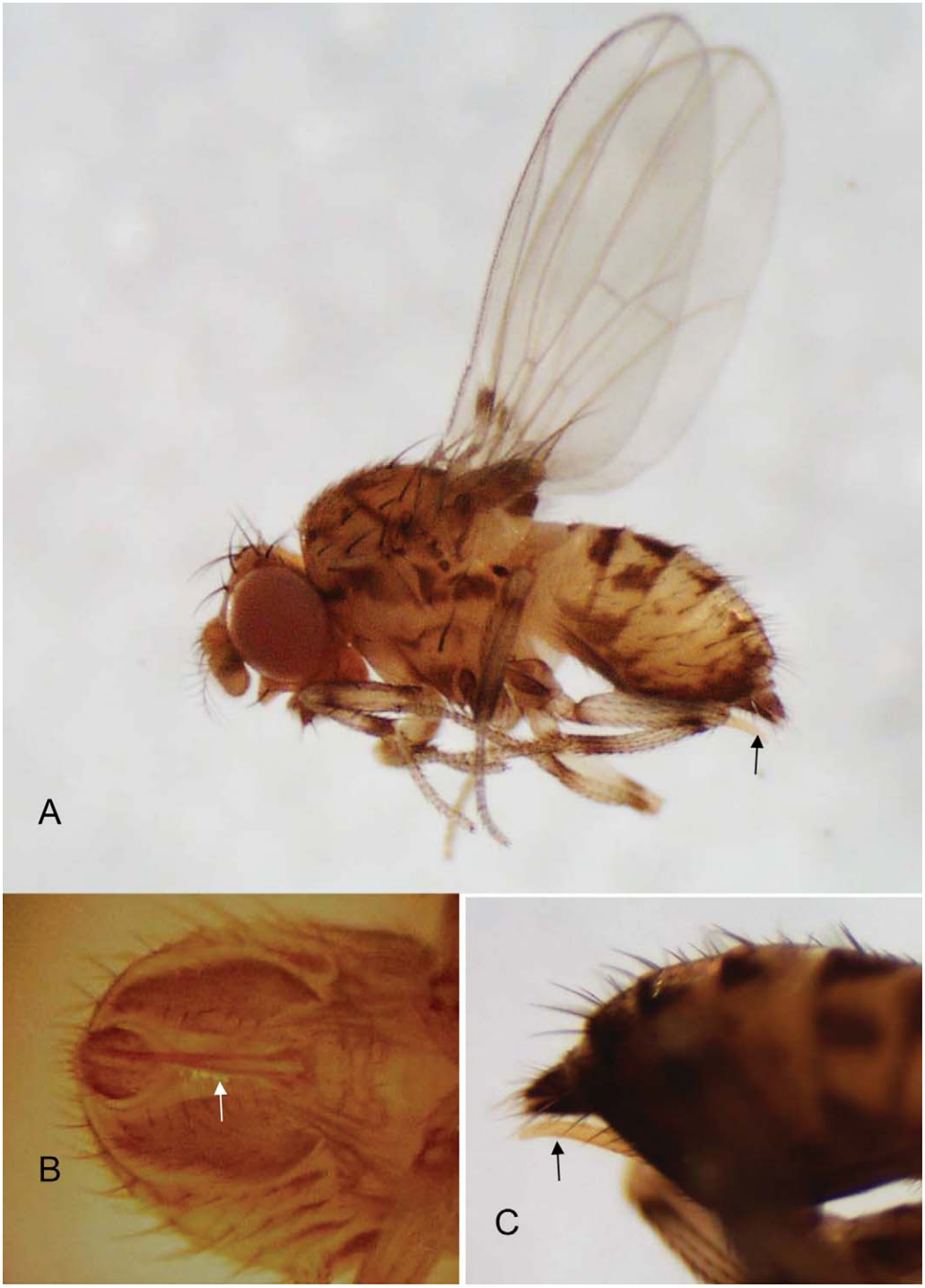

Fig. 5. *D. tessellata* eclosed from tepals of *A. crassipetala.* (A) Lateral view of adult female. (B) Ventral view of terminalia. (C) Lateral view of terminalia. The enlarged oviscape is indicated by an arrow in each view. (Online figure in color.)

These dual cohorts may represent an example of bet-hedging in this species because the adults that eclose from the first cohort are larger than those in the second cohort. If size is a reflection of reproductive fitness in this organism (e.g., Honek, 1993), then the smaller number of more fit individuals of the first cohort is being balanced by a larger number of potentially less fit individuals in the second cohort. The

factors that determine how females partition their oviposition behavior are unknown, but presumably the fleshy tepals of the open flower are more easily located and penetrated than the denser flower buds. In particular, the role of plant secondary compounds in this process is unknown.

A second intriguing observation is the skewed sex ratio of the reared flies (Fig. 4A). This ratio is significantly different from a canonical 1:1 ratio. Such ratios are not uncommon in drosophilids (Jaenicke 1996, 2001) and are often associated with meiotic drive mechanisms that favor production of female progeny (Tao et al. 2007a, 2007b). If such a mechanism underlies the observed female-biased ratio, it may have ecological value in structuring a population to optimally exploit a widely dispersed, limited, and ephemeral resource represented by these flowers.

Male flies have been observed displaying on the tepals of the open, odor-emitting flowers. Thus, the less common males could be easily found by females. After insemination the females could oviposit either on the tepals of open flowers and/or disperse to find immature buds for additional ovipositions.

This is the first record of flowers in Annonaceae being used as a larval substrate for drosophilids. However, although *A. crassipetala* has an unusually thick and fleshy whorl of tepals, it is not the only member of the family to have fleshy tepals. Given the ubiquity of Drosophilidae in the tropics, it is likely that many similar florivorous associations remain undetected. This prediction is supported by the fact that there are additional species of *Diathoneura* with prominent oviscapes (D. Grimaldi, personal communication). Furthermore, as this study was ending, similar or identical drosophilid larvae were observed within the fleshy tepals of *Xylopia bocatorena*, another understory tree in Annonaceae.

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