THE ECOLOGY OF HAWAIIAN FLOWER-BREEDING DROSOPHILIDS I. SELECTION IN THE LARVAL HABITAT

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Kambysellis and Heed (1971) first linked the host-plant preference of a drosophilid species to its specific compromise between egg size and number. They hypothesized that both the predictabilities (spatial and temporal) and the productivities (carrying capacities) of the larval substrates were critical selective factors in the evolution of reproductive strategies in the endemic Hawaiian drosophilid radiation. Mangan (1978), Atkinson (1979), and Lachaise (1983) presented convincing data from other drosophilid, host-plant associations that support this idea.

The six species of the endemic Hawaiian subgenus *Exalloscaptomyza* (Hardy 1965, 1966) lie at the extreme end of the large egg-small clutch compromise. These species oviposit in fresh morning glory blossoms (*Ipomoea* spp.). Carson et al. (1970) and Kambysellis and Heed (1971) noted that the *Exalloscaptomyza* species have the lowest fecundities (mature eggs per female) in the entire family. Whereas most drosophilid females so far recorded carry from 20 to over 100 relatively small mature eggs at a time, *Exalloscaptomyza* females carry only one or two relatively large eggs at a time.

Ibara (1976) and Montague and Kaneshiro (1982) reported morphological, behavioral, and ecological descriptions of these fascinating insects. Hawaiian morning glories remain open only for a single day, so the adult flies must redisperse each morning to fresh blossoms in order to court, mate, and lay eggs. The larvae develop within decaying blossoms. Kambysellis and Heed (1971) speculated that the larvae are pollen feeders, and that the low fecundities of the *Exalloscaptomyza* species evolved in response to severe, intraspecific larval competition for pollen.

In this paper, I report observations and experimental manipulations of the larval habitat of *Scaptomyza (Exalloscaptomyza) caliginosa* Hardy and *Drosophila (Phloridosa) floricola* Sturtevant, two species found breeding in morning glories in Hawaii Volcanoes National Park, on the big island of Hawaii. The results suggest that both density-dependent and density-independent stresses (larval crowding and moisture stress, respectively) have significantly negative effects on larval development and survivorship in the blossom habitat. The evolution of low

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fecundities in the morning glory drosophilids is then discussed as regards selection in the larval habitat.

METHODS AND MATERIALS

The research was completed during September-November 1979 and September-November 1980. The field site was Kipuka Puaulu ("Bird Park") near the Kilauea Crater in Hawaii Volcanoes National Park. Kipuka Puaulu is a mixed forest-savannah area roughly 35 km^2 in size. It lies 1,200 m above sea level on the southeastern slopes of the volcanic peak Mauna Loa.

Morning glory vines (*Ipomoea acuminata*) cover extensive areas of the open fields, and on any autumn morning, several tens of thousands of fresh morning glories bloom in Kipuka Puaulu (Montague 1982).

Field Estimates of Adults and Eggs per Blossom per Day

Montague and Kaneshiro (1982) described collection methods for the two species of morning glory drosophilids found breeding in Kipuka Puaulu. *Drosophila floricola*, a recently introduced exotic species, was found in low densities in 1979 and 1980.

Blossoms were randomly sampled from three selected sites in Kipuka Puaulu. Adults were caught by quickly enclosing plastic bags over the blossoms. Eggs per blossom were counted in the evening with a stereo-microscope. Developmental time for larvae was estimated from blossoms which were individually stored in perforated plastic bags containing sterile sand. As adults emerged they were collected and identified. The percent emerged per blossom (larval survivorship) was calculated by dividing the mean number emerged per blossom by the mean number of eggs per blossom.

Larval Development and Emergence from Control and Experimental Blossoms

Experimental manipulations of blossoms were completed during September-November 1979 and September-October 1980. All control and experimental blossoms were collected unopened at dawn and stored in cool, moist bags until midafternoon. In the afternoon, large numbers of fresh blossoms were then collected from the field and examined for eggs and first instar larvae. These were removed and placed in control and experimental blossoms in the desired quantities.

Pollen treatments (1979).—Variation in pollen was manipulated by the addition or removal of complete sets of anthers (*I. acuminata* blossoms have 5 anthers each).

Yeast treatments (1979).—Two types of yeasts were used in this design. Each was isolated and purified from Hawaiian morning glories by W. T. Starmer and J. R. Montague in August 1978 (see Starmer 1981). The isolates were mixed in sterile water and sprayed into selected experimental blossoms.

TABLE 1

ESTIMATES OF DENSITY (per blossom), LARVAL DEVELOPMENTAL TIME, AND PRE-ADULT SURVIVORSHIP FOR THE MORNING GLORY DROSOPHILIDS IN HAWAII VOLCANOES NATIONAL PARK

		Females per Blossom	Males per Blossom	Eggs per Blossom	Days to Emerge	Pre-adult Survivorship*
Scaptomyza caliginosa...	$\bar{x}$	2.10	1.89	1.48	12.9	.81
	SD	2.76	2.27	1.22	.91	
	N†	648	648	800	510	
Drosophila floricola	$\bar{x}$	.08	.05	.24	12.6	.56
	SD	.37	.30	.23	.73	
	N†	648	648	800	64	

*Survivorship = mean number of adults emerged per blossom/mean number of eggs per blossom.
†N = sample size (blossoms per sample or larvae per sample).

Density treatments (1979).—Density was set at two, four, and six larvae per blossoms. In both 1979 and 1980, the maximum number of eggs per blossom observed in the field was six.

All blossoms were individually stored in perforated plastic bags containing 1 g of sterile sand. After 3 days, a sterile wire loop was scraped over each blossom and then streaked onto plates of acidified Difco YM agar (pH = 3.5). These plates were examined for an additional 5 days for the presence of bacteria, molds, and yeasts. If yeasts were detected, the blossom in question was included in the "yeast present" category.

Density and moisture treatments (1980).—Larval density was set at one, four, and twelve larvae per blossom. After the addition of larvae, the blossoms were prepared as follows: (1) "dried blossoms" were stored with 1 g of silica gel added to the bag; (2) "wetted blossoms" were sprayed with sterile water, then sprayed again after 2 days; and (3) "unaltered blossoms" were used as controls.

In 1979 and 1980, all the bags were observed for 30 days. Emerged adults were identified, sexed, and measured with an ocular micrometer to determine thorax length.

Emerged thorax length, time to emergence, and percent emerged per blossom were used as variables in ANOVA. Time to emergence was transformed with a logarithmic function (ln [days]) to insure homogeneity of variances (after Sang 1956). Time to pupation could not be accurately determined because not all pupae were visible within the bags.

RESULTS

Field estimates of densities per blossom, and estimates of larval developmental time and survivorship are shown in table 1. *Scaptomyza caliginosa* adults and eggs were found in low densities in open blossoms, while *Drosophila floricola* densities were even lower. Adults of both species emerged approximately 13 days after oviposition, although a lower percentage of *D. floricola* larvae survived to emergence.

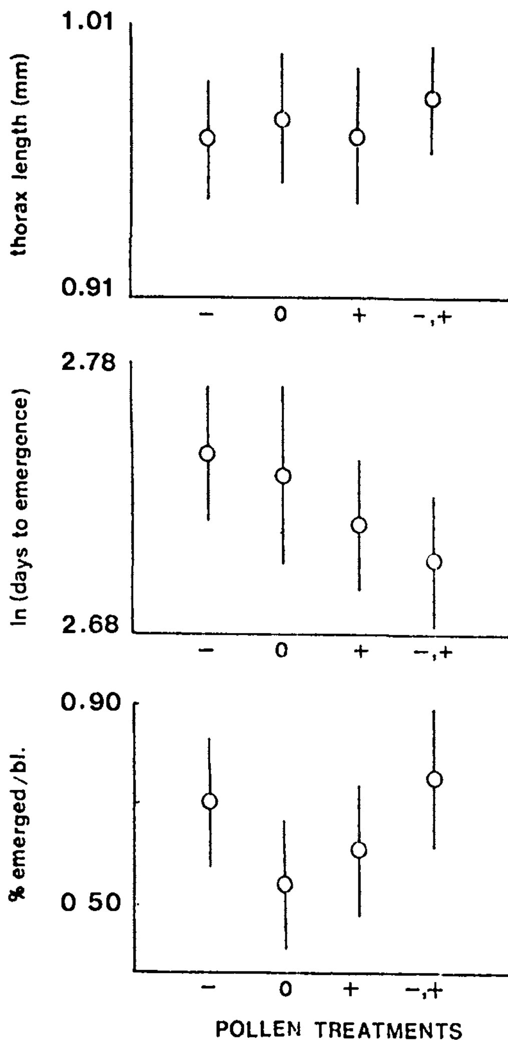

FIG. 1.—Mean thorax lengths, time to emergence, and percent emerged per blossom among pollen treatments: (–) = no pollen, (○) = pollen unaltered, (+) = pollen added, and (–, +) = pollen removed, then replaced. Circle and bar = ± 2 SE.

In 1979 and 1980, a total of 480 adults emerged from 153 control and experimental blossoms. Of these, only six were *D. floricola* (0.012 of the total). Wild adults of the two species are not significantly different in size or time to emergence (table 1; Montague and Kaneshiro 1982); it is therefore assumed that all the larvae used in the experimental manipulations were *S. caliginosa*.

1979 design.—These results are shown in figures 1, 2, and 3. The data were obtained from 162 adults reared from 72 blossoms. Although only 31 out of the 72 blossoms developed yeast florae, all of the blossoms developed bacterial florae.

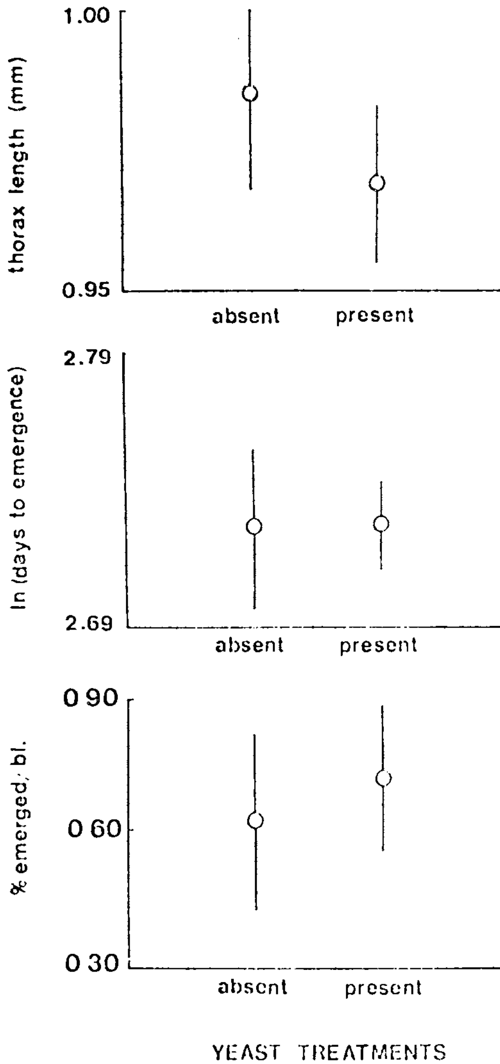

FIG. 2.—Mean thorax length, time to emergence, and percent emerged per blossom between yeast treatments (yeast present or absent). Circle and bar = ± 2 SE.

Emerged adult thorax length was not significantly affected by variation in pollen (P = .66; df = 3, 139), yeast (P = .13; df = 1, 139), or larval density (P = .09; df = 2, 139). There were no significant interactions among treatments.

Time to emergence was not significantly affected by variation in pollen (P = .13; df = 3, 139), yeast (P = .99; df = 1, 139), or larval density (P = .18; df = 2, 139). There were no significant interactions among treatments.

Percent emerged per blossom was not significantly affected by variation in pollen (P = .13; df = 3, 49), yeast (P = .27; df = 1, 49), or larval density (P = .09; df = 2, 49). There were no significant interactions among treatments.

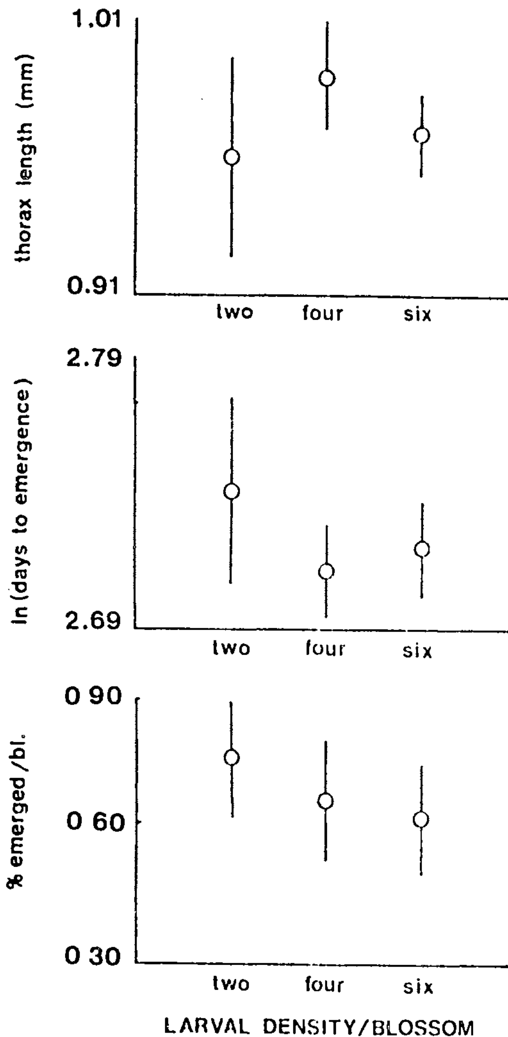

FIG. 3.—Mean thorax length, time to emergence, and percent emerged per blossom among larval density treatments. Circle and bar = ± 2 SE.

The 1979 results showed that within the observed range of field densities (≤ 6 larvae per blossom), the absence of pollen or yeasts had no significant effects on larval development or survivorship of *S. caliginosa*.

1980 design.—These results are shown in figure 4. The data were obtained from 318 adults reared from 81 blossoms.

Emerged adult size was significantly affected by variation in moisture ($p < .01$; df = 2, 310) and larval density ($P < .01$; df = 2, 310). The interaction among treatments was significant at the $\alpha = 0.06$ level (df = 4, 310): emerged adult sizes from the four per blossom and the twelve per blossom densities were not significantly different in the wetted treatments (fig. 4).

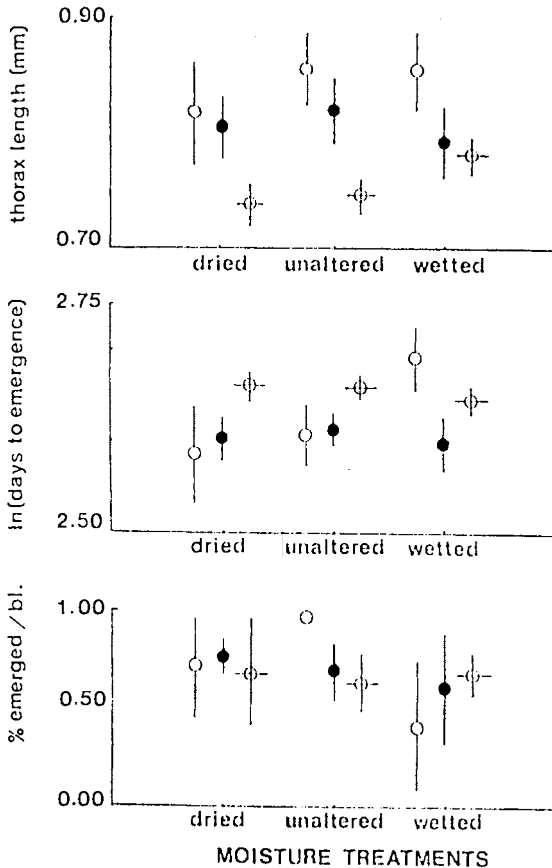

FIG. 4.—Mean thorax length, time to emergence, and percent emerged per blossom among density and moisture treatments. Open circle = 1 larva per blossom, closed circle = 4 larvae per blossom, and hatched circle = 12 larvae per blossom. Circle and bar = ± 2 SE.

Time to emergence was significantly affected by variation in larval density ($P < .01$; df = 2, 310) but not by variation in moisture ($P = .50$; df = 2, 310). There was significant interaction among treatments ($P = .04$; df = 4, 310): in the low density treatments (1 per blossom), adults took significantly longer to emerge from wetted treatments than from unaltered or dried treatments. This effect was not present in higher larval densities (fig. 4).

Percent emerged per blossom was significantly affected by variation in moisture ($P = .04$; df = 2, 72) but not by variation in larval density ($P = .83$; df = 2, 72). There was significant interaction among treatments at the $\alpha = 0.06$ level (df = 4, 72): adults from low density treatments (1 per blossom) emerged in the highest percentage from unaltered treatments (fig. 4). This effect was not present at higher larval densities.

The 1980 results showed that artificially high larval densities (twice the maximum field density per blossom) resulted in significantly delayed emergence and

stunted adult size. In low larval density treatments (1 per blossom), decreased larval survivorship was observed in blossoms that were either dried or wetted, though this effect was not apparent in higher larval densities.

DISCUSSION

Kambysellis and Heed (1971) hypothesized that severe larval competition for pollen resulted in the evolution of low fecundity and larval precocity in the *Exalloscaptomyza* species. There is evidence that larval crowding is a critical selective factor in the population dynamics of drosophilid species. In studies with *Drosophila melanogaster* (Giesel and Zettler 1980; Mueller and Ayala 1981) and *D. pseudoobscura* (Taylor and Condra 1980), *K*-selected laboratory populations (maintained for many generations under high larval densities) had significantly lower intrinsic rates of population growth than *r*-selected (low density) populations. But the large egg-small clutch strategy of the *Exalloscaptomyza* spp. might also have evolved through density-independent processes (e.g., strong or unpredictable variation in larval survivorship: Hastings and Caswell 1979; Wilbur 1980; Montague et al. 1981). Barclay and Gregory (1981) used laboratory populations of *D. melanogaster* to show that severe density-independent larval mortality resulted in *K*-selected characteristics (e.g., delayed maturity and increased survivorship). The results described in this paper indicate that both density-dependent and density-independent factors should be examined to understand the evolutionary forces thought to be responsible for the diversity of reproductive strategies in the Drosophilidae.

Carrying capacities of larval substrates.—The observed larval densities in morning glories in Kipuka Puaulu indicate that the blossoms cannot support more than six larvae per blossom. Experimental increases of density above this level resulted in delayed emergence and stunted adult size. Both of these characteristics are associated with severe larval crowding (Cannon 1966; Budnik and Brncic 1974; Atkinson 1979). Bakker (1969) and Burnet et al. (1977) reported that intraspecific larval competition in *D. melanogaster* selectively favors genotypes that produce rapidly growing larvae and early pupation.

The nutritional quality of the larval substrates should affect the larval carrying capacities. Most drosophilid substrates support yeast microflorae, and are considered to be nutritionally rich (Starmer 1981). On the other hand, decaying *Cheirodendron* leaves utilized by *D. dicticha* support bacterial growth with little or no yeasts, and are considered to be nutritionally limiting (Robertson et al. 1968). Mangan (1978) reported mean densities of less than four larvae per *Cheirodenron* leaf and suggested the low carrying capacity results from the poor nutritional quality available to larvae. Since *S. caliginosa* larvae appear to obtain nutrition from bacterial sources within blossoms (i.e., no yeast or pollen effects on development), they probably face nutritional constraints similar to those of the decaying leaf habitat.

The potential fecundity of a drosophilid species is limited by the number of ovarioles (egg chambers) carried by the females. Most drosophilid species have 20 to 60 ovarioles per female. Mangan (1978) reported that Hawaiian leaf-breeders

have between 10 and 20 ovarioles per female. Little is known about ovariole number in flower-breeding drosophilids. The *Exalloscaptomyza* have two to four ovarioles per female, and *D. floricola* has 12–14 per female (Montague and Kaneshiro 1982); Cook et al. (1977) reported that the Australian flower-breeding species *D. hibisci* has 10 ovarioles per female.

Brncic (1966) and Pipkin et al. (1966) examined 13 flower-breeding *Drosophila* species from Central and South America, and reported egg densities of one to two eggs per blossom (17 different blossom species were reported, including *Cestrum parqui*, and three species of *Heliconia*). Carson and Okada (1980) examined flower-breeding *Drosophilella* species from Papua, New Guinea, and reported egg densities of 15 to 90 eggs per blossom (*Colocasia esculenta*). Montague (1982), however, reported that Hawaiian morning glory blossoms are considerably smaller than *C. esculenta* blossoms, so they should support lower numbers of larvae. Carson and Okada (1980) also noted that the densities of eggs per female were relatively low within *C. esculenta* blossoms (1–4 eggs per female per blossom). More field studies of flower-breeding species might indicate whether these species show evolutionary convergence toward reduced ovariole number and increased egg size.

Density-independent selection in the larval habitats.—The effects of temperature and humidity stresses on dipteran species are poorly understood (Roff 1977, 1981; Collins 1980). Barker and Barker (1980) and Giesel et al. (1982) emphasized the difficulty of detecting the direction of density-independent selection on life-history traits of drosophilids. In *D. melanogaster*, emerged adult size is negatively correlated with substrate temperatures during larval development (Tantawy 1964; Atkinson 1979). The rates at which larval substrates deteriorate (or desiccate) may be inversely correlated with larval development time. The precise nature of temperature-humidity stresses within the larval habitat could not be determined in this study because there was no practical method available to control both temperature and humidity in the 1980 design.

Parsons (1978) reported that the relatively xeric, open-field habitat of the *Exalloscaptomyza* is subject to stronger seasonal variation in temperature and humidity than rain forest habitat. The experimental manipulations of morning glories indicated that blossoms that were either too dry or too moist yielded a significantly reduced percentage of adults (though this effect was not present at high larval densities). The large egg size and larval precocity of *S. caliginosa* appear to be adaptations that allow larvae to pass through the pre-adult stages as rapidly as possible.

SUMMARY

The population density estimates for the Hawaiian flower-breeding drosophilids indicate that morning glory blossoms can support only low numbers of larvae (≤ 6 per blossom). Evidence from experimental manipulations of blossoms indicates that *Scaptomyza caliginosa* larvae do not require pollen or yeasts in their diets. The nutritional requirements of *S. caliginosa* larvae appear to be qualitatively similar to those of Hawaiian leaf-breeding drosophilid larvae (i.e., bacterial mi-

croflorae within decaying plant tissues). The evidence also supports the notion that the large egg-small clutch strategy of *S. caliginosa* is an evolutionary response to both density-dependent (larval crowding) and density-independent (moisture stress) factors in the larval habitat.

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