

Phylogenetic, Geographical, and Temporal Analysis of Female Reproductive Trade-Offs in Drosophilidae

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The fact that reproductive effort often shows trade-offs with other necessary functions and features of living organisms has been recognized for centuries. Darwin (1872, pg. 142) gives credit to Geoffroy St. Hilaire and Goethe for proposing the law of "Compensation or Balancement of Growth" and ascribes the following quote to Goethe, "In order to spend on one side, nature is forced to economize on the other side." The essence of this law is captured in modern theories and syntheses (Lack, 1947; Cody, 1966; Smith and Fretwell, 1974; Stearns, 1976, 1977, 1992) that emphasize

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time, energy budgets, and physiological, genetic and phylogenetic associations that govern the form of compensation that results in a trade-off.

One trade-off that is expected to be widespread is between offspring size and number. Particularly in species with no parental care, it is presumed that offspring viability increases with egg size (e.g., Smith and Fretwell, 1974; Parker and Begon, 1986; McGinley et al., 1987; Winkler and Wallin, 1987). Because energy allocated to reproduction must be divided among offspring, any increase in maternal resources devoted to each offspring should diminish the number of offspring produced. Indeed, a phenotypic trade-off between egg size and fecundity, indicative of such an underlying physiological trade-off, has been widely identified (e.g., Darwin, 1872; Lack, 1947; Stearns, 1992). To the extent that population-level responses to selection on genetic variation in this physiological trade-off occur, a microevolutionary (i.e., genetic) trade-off between egg size and number is expected. Finally, if conditions relevant to this trade-off are common to whole lineages, then a macroevolutionary trade-off between these traits may result.

In the genus *Drosophila*, the relationship between egg size and fecundity is unclear. Studies of three species (*D. simulans*, *D. subobscura* and *D. phalerata*) found no evidence of intraspecific phenotypic trade-offs between egg volume and egg number (Avelar and Rocha Pit e, 1989; Avelar, 1993). An experimental study imposing bidirectional selection on egg size in *D. melanogaster* found a phenotypic correlation between egg size and fecundity in the lines selected for increased egg size, but not in the control or decreased egg size lines. No genetic correlation between egg size and fecundity was observed (Schwarzkopf et al., 1999). In contrast, significant negative phenotypic relationships between egg size and fecundity have been reported among populations of *D. hibisci* (Starmer et al., 1997; Wolf et al., 2000) and among species of Hawaiian *Drosophila* (Montague et al., 1981; Berrigan, 1991).

The expected negative relation between these traits can be expressed as a power function such that egg size = $\alpha \times (\text{egg number})^\beta$. The exponent β is expected to be equal to -1 when the trade-off is isometric, e.g. egg size \times egg number is constant (α). In the Hawaiian drosophilids, which have egg sizes ranging from 0.014 to 0.269 mm³ and clutch sizes from 2 to 150 (Kambyzellis and Heed, 1971), β was estimated to be close to -1 depending on assumptions about ovariole activity responsible for egg number data (Montague et al., 1981). The extraordinary situation responsible for the evolution of the Hawaiian fauna (Carson and Kaneshiro, 1976) and the extreme isolation of this radiation, however, make the relationship among egg size, egg number and reproductive effort dynamics somewhat singular. It is thus desirable to compare the Hawaiian example to other drosophilid radiations of similar magnitude.

To better elucidate this important trade-off in Drosophilidae, we here determine the relationship between egg size and fecundity at multiple levels: temporal, geographic, interspecific, intergeneric, and among three discrete continental radiations. We use field data from the flower-breeding *Scaptodrosophila hibisci* (*Drosophila hibisci*) collected over a wide geographic range and over time at the same location in eastern Australia (Starmer et al., 1997, 1998, 2000; Wolf et al., 2000). Laboratory isofemale lines from this species collected at separate localities (Starmer et al., 2000) provide additional information on the intrapopulation level of variation for the trade-off and for reproductive effort. In addition, specific comparisons of field-collected versus laboratory-reared females from the same site, provide information about the laboratory effects on reproductive activity. Recently, *Scaptodrosophila acinata*, a species closely related to *S. hibisci*, was discovered in the Northern Territory (McEvey and Barker, 2001). Data from this species are included to provide a comparison between two closely related species. Intergeneric comparisons are also examined. Finally, we compare the egg size-egg number relationship among three discrete radiations.

In addition to the Drosophilidae of Hawaii, Carson and Okada (1982), Bock (1976) and Parsons and Bock (1979) have implicated the *Scaptodrosophila* of New Guinea and Australia as another example of a large adaptive radiation. Some North American species of *Drosophila* have been investigated in conjunction with the earlier Hawaiian work (Berrigan, 1991) and a study of domestic drosophilids (Atkinson, 1979) in Europe has been reported, but comparisons between the continental and island groups have not been made. The Drosophilidae species in these three geographic regions (Hawaiian, Australasian and North American) differ in several respects. The salient differences are: 1) the geographic setting is largest for the continental North American fauna, intermediate for the Australasian and smallest for the Hawaiian; 2) the adult body size variation is much larger for the Hawaiian flies; 3) the time scale of evolution for the Hawaiian forms is shorter; 4) the fauna in Hawaii is representative of a single radiation, whereas the North American taxa include Nearctic and Neotropical components; and 5) although not entirely known, the habitats of the Hawaiian species are more varied. All of these differences can be used to argue that the functional relationship between egg number and egg size may not be the same for the three regions. Indeed, movement away from an isometric relationship and changes in reproductive effort can implicate both adaptive and non-adaptive constraints such as phylogenetics, demands on locomotor performance (Berrigan, 1991), minimal egg size (Wiklund et al., 1987), and allometric growth differences due to environmental differences.

MATERIALS AND METHODS

Data for Hawaiian taxa were taken from Kambysellis and Heed (1971). The Australasian data were collected from wild-caught females that were attracted to baits, found on natural substrates or swept from foliage and litter. These collections were made in four regions (Tasmania, central New South Wales, northern Queensland and southern New Caledonia) during January and February, 2000. Records for *S. hibisci* and *S. acclinata* were from extensive collections over the species ranges prior to the 2000 collection (Starmer et al., 1997, 1998, 2000, and Wolf et al., 2000). The North American data were mainly gathered from laboratory stock cultures obtained from the Bowling Green stock center or from our stocks. Five species (*Drosophila neotestacea*, *D. putrida*, *Hirtodrosophila duncani*, *Mycodrosophila claytonae*, *M. dimidiata*) were collected from wild mushrooms in central New York state in the summer of 2000. Female size (thorax length = **tl**), ovariole number (**ova**) and egg dimensions (egg width = **ew**, egg length = **el**) were determined by the methods described in Starmer et al. (1997, 1998). Egg volume (**ev**) was calculated as a prolate spheroid, $ev = (1/6)\pi \times ew^2 \times el$. Relative egg volume (**rev**) is the ratio of **ev** to **tl**³, $rev = ev/tl^3$.

Two related expressions use egg size, egg number and body size to (1) calculate *relative* reproductive effort (**rev** × **ova**) and (2) model the *absolute* reproductive effort as a function of thorax length

$$rev \times ova = \frac{ev \times ova}{tl^3} \quad (1)$$

and

$$ev \times ova = \alpha \times tl^\beta \quad (2)$$

In the first expression the scaling relationship obtained by dividing reproductive volume by thorax length (**tl**³ = fly volume) is assumed to result in a non-dimensional factor (volume/volume), i. e., relative reproductive allocation (**rev** × **ova**). If the power = 3 for **tl** assumption is relaxed, then equation (1) can be modified to model constant reproductive effort as a power function of thorax length (**tl**).

The trade-off between egg size and egg number can be modeled as a power function where relative egg volume (**rev**) is a function of ovariole number (**ova**),

$$rev = \alpha \times ova^\beta \quad (3)$$

and

$$\mathbf{ev} = \alpha \times \mathbf{ova}^{\beta_0} \times \mathbf{tl}^{\beta_1} \quad (4)$$

Similar to the conceptual connection of expression (1) and Model 2 the scaling relationship for female body size is assumed to be \mathbf{tl}^3 (volume/volume) in Model 3 or can be treated as a fitted constant (β_1) in Model 4. Model 3 and expression (1) are directly related when the trade-off between egg size and ovariole number is isometric. In this case $\beta_0 = -1$ and the constant (α) of Model 3 is equal to $\mathbf{rev} \times \mathbf{ova}$.

The models expressed in (2), (3) and (4) can be transformed to linear forms by taking logarithms of both sides of the equations.

$$\log(\mathbf{ev} \times \mathbf{ova}) = \log(\alpha) + \beta_1 \times \log(\mathbf{tl}), \quad (5)$$

$$\log(\mathbf{rev}) = \log(\alpha) + \beta_0 \times \log(\mathbf{ova}), \quad (6)$$

and

$$\log(\mathbf{ev}) = \log(\alpha) + \beta_0 \times \log(\mathbf{ova}) + \beta_1 \times \log(\mathbf{tl}). \quad (7)$$

These linear equations express the relationship between reproductive volume ($\mathbf{ev} \times \mathbf{ova}$), relative egg volume (\mathbf{rev}), and egg volume (\mathbf{ev}) as respective functions of thorax length (\mathbf{tl}), ovariole number (\mathbf{ova}), or both \mathbf{ova} and \mathbf{tl} . When the relationships are used in analysis of covariance, regional, intergeneric, interspecific, intraspecific geographic and intraspecific temporal variability in reproductive activity can be investigated.

Comparisons of estimates of $\mathbf{rev} \times \mathbf{ova}$ and β_0 and β_1 were made for geographic regions and for genera, using data of each species. *Scaptodrosophila hibisci* and *S. acclinata* data were used to evaluate closely related species, intraspecific geographic, temporal, among isofemale line, and laboratory versus field variability. The laboratory versus field comparisons were made by collecting females of *S. hibisci* from one site (Bellingen, N.S.W.) over a two week period in the spring of two successive years (1996 and 1997). Females and new flowers with eggs were collected and returned to the laboratory. The field females were dissected immediately. The flowers were placed on damp sand in jars and incubated at different temperatures. Females emerging from the flowers were allowed to mature in cages with fresh *Hibiscus heterophyllus* blossoms before dissection.

Comparisons for Family, Regions or Genera, and Species used genus means, species means, and population means, respectively, while intraspecific analysis employed site, population or isofemale-line means of *S. hibisci*.

Only species statistics were used for the Hawaiian data because these values were obtained from the literature (Kambysellis and Heed, 1971) and individual data were not available. Because the North American species were mainly from laboratory populations, with low sample sizes, only species means were used for analysis. Throughout the analysis, the classification of genera proposed by Grimaldi (1990) was followed.

ANOVAs (SAS, Proc GLM) employed Type III sums of squares with all nested components considered to be random effects. In all regression analyses least-squares estimation procedures (SAS, Proc REG) were used to estimate β_0 and β_1 and the corresponding r^2 for each model expressed in equations (2), (3) and (4). Reduced major axis estimates of the functional relationship (Rayner, 1985) can be derived from the statistics presented in the tables and results.

Before comparative examination of evolutionary relationships between characters, it is preferable to control for phylogenetic effects (Felsenstein, 1985; Harvey and Pagel, 1991). Because phylogenetic relationships are unknown for the Hawaiian and Australasian taxa, character relationships were examined and compared across several hierarchical levels. Additionally for the North American taxa, Felsenstein's (1985) method of phylogenetically independent contrasts was employed, which provides statistical independence of data points. Independent contrasts were computed (using the phylogenetic topology and branch lengths presented in Figs. 2 and 3 of Pitnick et al., 1999) using the Comparative Analysis by Independent Contrasts (CAIC) program of Purvis and Rambaut (1995). The analyses presented employ a model that assumes gradual evolutionary change in variables, with branch lengths equal to estimated times of divergence (Felsenstein, 1985).

The phylogeny was compiled from a number of sources. The higher level relationships were inferred from several published morphological (Grimaldi et al., 1992; Throckmorton, 1962, 1975) and molecular (Beverley and Wilson, 1982, 1984; Spicer, 1988; Sullivan et al., 1990; Caccone et al., 1992; DeSalle, 1992; Pelandakis and Solignac, 1993; Kwiatowski et al., 1994; Russo et al., 1995; Powell and DeSalle, 1995; Powell, 1997) data sets. In addition to the published sources, an unpublished data set consisting of 2.7 Kb of nuclear large-subunit (28S) ribosomal RNA sequence was used (C. Bell, C. Saux, and G. S. Spicer, unpublished). The lower level relationships were determined both from published sources and from unpublished DNA sequences comprising about 1.5 Kb of the mitochondrial cytochrome oxidase subunits (G. S. Spicer, unpublished). Phylogenetic relationships for the *D. melanogaster* (Ashburner, 1989) and *D. quinaria* (Spicer and Jaenike, 1996) species groups were inferred entirely from the literature, whereas the relationships within the *D. virilis* (Spicer, 1991, 1992) and *D. repleta*

(Wasserman, 1992; Spicer and Pitnick, 1996) species groups were determined by using a combination of published phylogenies and the unpublished sequencing studies. Relationships within the *D. nanoptera* and *D. melanica* species groups were inferred entirely from unpublished sequencing studies.

RESULTS

The species used in the geographic and generic comparisons and associated statistics for thorax length, ovariole number, egg width and egg length are listed in Table 1. The means in Table 1 were used to estimate regional statistics (mean and coefficient of variation, CV) for thorax length, ovariole number and egg volume (Table 2). Comparisons of these regional means show that the Hawaiian region has the largest CVs for all variables, and has the largest mean thorax length and egg volume. The other two geographic regions have similar but smaller mean egg volumes and thorax lengths. The Australasian region has greater variation for ovariole number and egg volume but similar variability for thorax size, when compared to the North American region. However, the means of all three variables for Australasian and North American regions are not statistically different ($\alpha = 0.05$). The only mean that is similar across all three regions is mean ovariole number.

The means in Table 1 also were used to estimate means and CVs for the six genera for which more than one species was examined (Table 2). Analysis of variance shows that all variables (**tl**, **ova** and **ev**) are significantly different among genera. Species belonging to the genus *Idiomyia* are larger, whereas members of the *Scaptomyza* have fewer ovarioles and larger eggs than members of the other genera. The regional differences in body and egg size, setting Hawaii apart from the other regions, is primarily a result of the fact that *Idiomyia* and *Scaptomyza* are restricted to the Hawaiian fauna.

The range in absolute egg size for the species from all regions (Fig. 1) is almost 100× with eggs of *Mycodrosophila variata* from New Caledonia being the smallest (0.0029 mm^3) and eggs of *Scaptomyza undulata* from Hawaii being the largest (0.2691 mm^3). The range in egg size within regions is smaller (18.6× for Hawaiian, 13.3× for Australasian and 3.2× for North American taxa). The range in relative egg size (**REV**) is even more pronounced for the Hawaiian species (93.4×), whereas **REV** for the other two regions is similar to the ranges in absolute egg size (11.1× for Australasia and 4.3× for North America).

TABLE 1. Geographic Region (R), Genus(G), Species, Mean \pm std: Thorax Length (tl, mm), Ovariole Number (ova), Egg Width (ew, mm) and Egg Length (el, mm)

R	G	species	n	tl \pm std	ova \pm std	n	ew \pm std	el \pm std
ha	Sz	<i>caliginosa</i>	24	0.93 \pm 0.078	2.50 \pm 0.293	10	0.32 \pm 0.029	0.88 \pm 0.016
ha	Sz	<i>mauiensis</i>	18	0.87 \pm 0.034	4.00 \pm 0.000	10	0.28 \pm 0.015	0.74 \pm 0.022
ha	Sz	<i>oahuensis</i>	19	0.86 \pm 0.042	4.05 \pm 0.250	15	0.25 \pm 0.016	0.73 \pm 0.033
ha	Sz	<i>throckmortoni</i>	29	0.84 \pm 0.059	4.07 \pm 0.253	15	0.28 \pm 0.014	0.73 \pm 0.034
ha	Sz	<i>undulata</i>	6	2.00 \pm 0.078	2.00 \pm 0.000	15	0.71 \pm 0.015	1.02 \pm 0.023
ha	Sz	<i>nasalis</i>	22	1.90 \pm 0.342	4.68 \pm 1.660	15	0.48 \pm 0.013	1.41 \pm 0.038
ha	Sz	<i>reducta</i>	4	1.68 \pm 0.150	5.33 \pm 1.110	0	.	.
ha	Sz	<i>crassifemur</i>	14	2.06 \pm 0.292	5.36 \pm 0.902	15	0.39 \pm 0.019	1.01 \pm 0.091
ha	Sz	<i>inflatus</i>	3	1.69 \pm 0.025	7.25 \pm 0.213	0	.	.
ha	Id	<i>villosus</i>	5	2.92 \pm 0.087	8.40 \pm 0.456	30	0.47 \pm 0.017	1.66 \pm 0.063
ha	Id	<i>aduncus</i>	21	2.94 \pm 0.119	11.00 \pm 1.233	30	0.46 \pm 0.022	1.61 \pm 0.039
ha	Id	sp	10	2.33 \pm 0.129	14.30 \pm 0.642	25	0.37 \pm 0.012	1.25 \pm 0.033
ha	Id	<i>diamphidiopodus</i>	18	2.28 \pm 0.131	18.44 \pm 1.065	25	0.29 \pm 0.016	1.09 \pm 0.039
ha	Id	<i>prodita</i>	12	1.37 \pm 0.138	9.00 \pm 2.311	0	.	.
ha	Id	<i>trichetosa</i>	9	1.35 \pm 0.096	10.33 \pm 1.632	0	.	.
ha	Id	<i>disticha</i>	244	1.45 \pm 0.101	11.79 \pm 1.532	30	0.26 \pm 0.008	0.90 \pm 0.028
ha	Id	<i>pectinitarsus</i>	12	1.26 \pm 0.028	12.42 \pm 1.604	25	0.22 \pm 0.007	0.69 \pm 0.033
ha	Id	<i>kambysellisi</i>	26	1.51 \pm 0.076	15.00 \pm 1.226	30	0.23 \pm 0.007	0.79 \pm 0.021
ha	Id	<i>petalopeza</i>	16	1.75 \pm 0.072	17.75 \pm 1.820	0	.	.
ha	Id	<i>mimica</i>	325	1.78 \pm 0.184	23.85 \pm 4.214	39	0.22 \pm 0.006	0.74 \pm 0.020
ha	Id	<i>primaeva</i>	7	3.00 \pm 0.124	101.33 \pm 8.439	30	0.23 \pm 0.006	0.83 \pm 0.021
ha	Id	<i>attigua</i>	2	2.67 \pm 0.063	43.00 \pm 1.003	27	0.22 \pm 0.009	0.81 \pm 0.023
ha	Id	<i>setosimentum</i>	33	2.13 \pm 0.086	35.61 \pm 3.889	24	0.23 \pm 0.004	0.86 \pm 0.021
ha	Id	<i>adiastola</i>	21	2.41 \pm 0.092	45.92 \pm 7.246	25	0.23 \pm 0.006	0.82 \pm 0.023
ha	Id	<i>truncipenna</i>	6	3.22 \pm 0.129	48.00 \pm 4.896	25	0.25 \pm 0.090	0.96 \pm 0.035

ha	Id		7	2.71 ± 0.114	38.17 ± 3.558	25	0.31 ± 0.010	0.99 ± 0.031
ha	Id	<i>clavisetae</i>	9	1.77 ± 0.098	27.44 ± 3.654	33	0.24 ± 0.011	0.81 ± 0.026
ha	Id	<i>picticornis</i>	6	3.31 ± 0.342	86.60 ± 9.243	30	0.22 ± 0.007	0.90 ± 0.034
ha	Id	<i>melanocephala</i>	13	3.16 ± 0.129	52.38 ± 2.672	36	0.25 ± 0.015	0.94 ± 0.030
ha	Id	<i>silvestris</i>	5	2.98 ± 0.305	53.33 ± 1.886	24	0.24 ± 0.007	1.04 ± 0.024
ha	Id	<i>nigribasis</i>	8	2.19 ± 0.167	45.00 ± 3.739	25	0.22 ± 0.035	0.75 ± 0.014
ha	Id	<i>pilimana</i>	9	2.65 ± 0.222	47.22 ± 6.357	25	0.22 ± 0.007	0.77 ± 0.023
ha	Id	<i>fasciculisetae</i>	10	2.30 ± 0.136	34.00 ± 3.267	30	0.23 ± 0.011	0.92 ± 0.012
ha	Id	<i>punalua</i>	16	2.09 ± 0.188	40.00 ± 4.288	30	0.21 ± 0.008	0.81 ± 0.021
ha	Id	<i>crucigera</i>	13	2.48 ± 0.115	59.73 ± 7.448	24	0.23 ± 0.014	0.87 ± 0.036
ha	Id	<i>engyochracea</i>	42	2.78 ± 0.149	65.55 ± 5.929	24	0.19 ± 0.008	0.87 ± 0.020
ha	Id	<i>sproati</i>	7	2.24 ± 0.212	56.83 ± 5.895	21	0.26 ± 0.023	0.97 ± 0.021
ha	Id	<i>sejuncta</i>	5	2.40 ± 0.096	38.00 ± 4.595	20	0.21 ± 0.007	1.09 ± 0.020
ha	Id	<i>ochracea</i>	21	2.43 ± 0.119	41.57 ± 4.798	24	0.21 ± 0.008	0.91 ± 0.013
ha	Id	<i>murphyi</i>	6	2.09 ± 0.144	47.00 ± 3.633	30	0.22 ± 0.006	0.87 ± 0.028
ha	Id	<i>villosipedis</i>	2	1.20 ± .	37.50 ± .	15	0.20 ± .	0.69 ± .
ha	Id	<i>preapicala</i>	6	0.86 ± 0.053	23.00 ± 3.347	2	0.15 ± 0.003	0.48 ± 0.014
au	Dr	<i>ananasse</i>	4	0.92 ± 0.058	25.00 ± 1.414	3	0.15 ± 0.002	0.45 ± 0.020
au	Dr	<i>bipectinata</i>	6	0.95 ± 0.086	13.33 ± 3.777	5	0.19 ± 0.013	0.56 ± 0.021
au	Dr	<i>fcusphila</i>	4	0.82 ± 0.075	22.00 ± 3.559	1	0.17 ± .	0.53 ± .
au	Dr	<i>flavohirta</i>	6	1.26 ± 0.068	27.50 ± 2.168	5	0.20 ± 0.008	0.57 ± 0.046
au	Dr	<i>pseudotetrachaeta</i>	6	1.24 ± 0.096	37.67 ± 5.715	2	0.18 ± 0.000	0.58 ± 0.003
au	Dr	<i>sulfurigaster</i>	6	1.31 ± 0.088	36.00 ± 4.427	2	0.20 ± 0.003	0.53 ± 0.008
au	Hi	<i>hannae</i>	3	1.10 ± 0.047	24.00 ± 5.568	1	0.18 ± .	0.56 ± .
au	Hi	<i>hirudo</i>	1	1.13 ± .	24.00 ± .	1	0.17 ± .	0.40 ± .
au	Hi	<i>laurelae</i>	6	1.11 ± 0.125	34.17 ± 7.910	1	0.18 ± .	0.43 ± .
au	Hi	<i>mixtura.1</i>	3	1.07 ± 0.114	24.33 ± 2.887	0
au	Hi	<i>polypori</i>	2	1.14 ± 0.042	6.50 ± 0.707	1	0.30 ± .	0.80 ± .
au	Hi	<i>trifurca</i>	6	0.93 ± 0.094	22.00 ± 4.427	3	0.19 ± 0.010	0.46 ± 0.033
au	My	<i>boudinoti</i>	7	0.73 ± 0.043	24.14 ± 4.706	2	0.12 ± 0.000	0.41 ± 0.000
au	My	<i>cf.boudinoti</i>						

Continued

TABLE 1. Continued.

R	G	species	n	tl ± std	ova ± std	n	ew ± std	el ± std
au	My	<i>cf. minor</i>	8	1.06 ± 0.060	28.25 ± 1.909	3	0.15 ± 0.013	0.45 ± 0.015
au	Mi	<i>elator</i>	4	0.81 ± 0.039	22.25 ± 2.500	2	0.14 ± 0.011	0.43 ± 0.017
au	My	<i>minor</i>	4	1.11 ± 0.055	27.25 ± 5.620	6	0.17 ± 0.005	0.48 ± 0.016
au	Zy	<i>samoensis</i>	6	1.04 ± 0.117	27.50 ± 4.278	2	0.18 ± 0.011	0.54 ± 0.022
au	Sc	<i>bryani</i>	3	0.85 ± 0.051	71.33 ± 9.238	3	0.13 ± 0.005	0.35 ± 0.018
au	Sc	<i>collessi</i>	5	1.10 ± 0.062	29.80 ± 3.962	1	0.17 ± .	0.55 ± .
au	Sc	<i>eluta</i>	8	0.98 ± 0.062	32.75 ± 5.092	2	0.17 ± 0.011	0.44 ± 0.006
au	Sc	<i>brunnea</i>	3	1.46 ± 0.047	64.33 ± 5.686	5	0.18 ± 0.010	0.49 ± 0.027
au	Sc	<i>fumida</i>	4	1.06 ± 0.064	45.25 ± 5.679	2	0.16 ± 0.006	0.46 ± 0.008
au	Sc	<i>fuscithorax</i>	1	0.98 ± .	37.00 ± .	0	.	.
au	Sc	<i>inornata</i>	3	1.04 ± 0.088	47.67 ± 13.80	3	0.18 ± 0.008	0.53 ± 0.016
au	Sc	<i>lattivittata</i>	6	1.20 ± 0.077	36.50 ± 4.930	3	0.19 ± 0.005	0.53 ± 0.008
au	Sc	<i>novoguineensis</i>	5	1.25 ± 0.127	74.40 ± 9.529	2	0.14 ± 0.000	0.44 ± 0.017
au	Sc	<i>rhabdote</i>	1	1.14 ± .	38.00 ± .	0	.	.
au	Sc	<i>zophera</i>	8	1.01 ± 0.083	47.13 ± 8.806	2	0.14 ± 0.011	0.45 ± 0.028
au	Sc	<i>Scapto.b</i>	6	0.99 ± 0.111	45.83 ± 8.134	1	0.15 ± .	0.43 ± .
au	Sc	<i>hibisci</i>	787	0.91 ± 0.098	14.60 ± 4.548	443	0.21 ± 0.020	0.66 ± 0.050
au	Sc	<i>acclinata</i>	286	0.77 ± 0.083	13.85 ± 3.757	181	0.20 ± 0.015	0.63 ± 0.037
na	Dr	<i>floricola</i>	46	0.88 ± 0.073	14.07 ± 1.855	30	0.19 ± 0.012	0.59 ± 0.029
na	Dr	<i>repleta</i>	8	1.32 ± 0.033	36.50 ± 3.295	8	0.16 ± 0.005	0.52 ± 0.022
na	Dr	<i>arizonae</i>	10	1.05 ± 0.025	34.60 ± 2.413	6	0.15 ± 0.008	0.46 ± 0.019
na	Dr	<i>mettleri</i>	8	1.20 ± 0.030	44.75 ± 1.832	7	0.14 ± 0.003	0.49 ± 0.010
na	Dr	<i>micromettleri</i>	7	1.14 ± 0.010	36.29 ± 2.563	4	0.17 ± 0.007	0.46 ± 0.017
na	Dr	<i>mojavensis</i>	6	1.03 ± 0.029	33.17 ± 2.639	7	0.15 ± 0.005	0.46 ± 0.011
na	Dr	<i>acanthoptera</i>	8	1.15 ± 0.028	41.88 ± 2.949	8	0.16 ± 0.004	0.53 ± 0.014
na	Dr	<i>nannoptera</i>	10	1.06 ± 0.026	37.60 ± 1.647	6	0.17 ± 0.009	0.48 ± 0.018

na	Dr	<i>wassermani</i>	8	1.15 ± 0.028	33.88 ± 2.949	8	0.16 ± 0.002	0.50 ± 0.011
na	Dr	<i>pachea</i>	6	0.99 ± 0.045	28.17 ± 2.317	5	0.16 ± 0.007	0.45 ± 0.022
na	Dr	<i>americana</i>	10	1.29 ± 0.024	31.60 ± 2.716	10	0.17 ± 0.005	0.54 ± 0.013
na	Dr	<i>borealis</i>	9	1.29 ± 0.036	30.78 ± 1.563	9	0.18 ± 0.008	0.53 ± 0.023
na	Dr	<i>lummei</i>	8	1.42 ± 0.035	36.00 ± 1.927	7	0.18 ± 0.005	0.57 ± 0.009
na	Dr	<i>virilis</i>	14	1.25 ± 0.041	41.21 ± 2.914	14	0.17 ± 0.006	0.56 ± 0.019
na	Dr	<i>laticola</i>	6	1.22 ± 0.029	28.67 ± 1.211	4	0.16 ± 0.007	0.49 ± 0.006
na	Dr	<i>montana</i>	10	1.32 ± 0.027	28.80 ± 1.476	8	0.18 ± 0.004	0.58 ± 0.015
na	Dr	<i>texana</i>	5	1.27 ± 0.032	38.00 ± 2.739	5	0.16 ± 0.002	0.51 ± 0.010
na	Dr	<i>eohydei</i>	9	1.28 ± 0.041	39.22 ± 3.667	9	0.16 ± 0.007	0.53 ± 0.021
na	Dr	<i>nigrohydei</i>	6	1.34 ± 0.041	47.33 ± 5.203	6	0.17 ± 0.005	0.52 ± 0.026
na	Dr	<i>hydei</i>	8	1.43 ± 0.054	51.75 ± 4.268	8	0.17 ± 0.006	0.53 ± 0.019
na	Dr	<i>bifurca</i>	15	1.53 ± 0.052	51.53 ± 4.454	13	0.17 ± 0.009	0.54 ± 0.015
na	Dr	<i>robusta</i>	4	1.47 ± 0.048	41.25 ± 4.573	3	0.16 ± 0.002	0.53 ± 0.003
na	Dr	<i>immigrans</i>	8	1.48 ± 0.044	65.38 ± 5.041	8	0.16 ± 0.007	0.53 ± 0.012
na	Dr	<i>micromelanica</i>	7	1.16 ± 0.018	25.57 ± 1.902	5	0.18 ± 0.006	0.51 ± 0.016
na	Dr	<i>melanica</i>	5	1.33 ± 0.014	42.20 ± 1.095	3	0.20 ± 0.005	0.50 ± 0.019
na	Dr	<i>neotestacae</i>	5	1.06 ± 0.069	36.60 ± 3.647	5	0.16 ± 0.006	0.46 ± 0.014
na	Dr	<i>putrida</i>	2	1.04 ± 0.011	40.00 ± 1.414	2	0.15 ± 0.006	0.45 ± 0.025
na	Dr	<i>subpalustris</i>	5	1.35 ± 0.016	26.20 ± 1.095	5	0.20 ± 0.005	0.54 ± 0.011
na	Dr	<i>melanogaster</i>	7	0.98 ± 0.036	33.14 ± 1.345	7	0.18 ± 0.007	0.49 ± 0.022
na	Dr	<i>simulans</i>	6	0.89 ± 0.024	36.83 ± 2.563	7	0.17 ± 0.003	0.49 ± 0.013
na	Dr	<i>willistoni</i>	5	0.90 ± 0.030	22.60 ± 1.673	5	0.17 ± 0.008	0.48 ± 0.022
na	Dr	<i>pseudoobscura</i>	12	1.09 ± 0.057	45.42 ± 5.915	7	0.16 ± 0.006	0.46 ± 0.016
na	Dr	<i>busckii</i>	7	0.98 ± 0.081	52.86 ± 9.771	4	0.13 ± 0.008	0.38 ± 0.012
na	Hi	<i>duncani</i>	6	1.17 ± 0.037	47.00 ± 8.075	5	0.16 ± 0.006	0.48 ± 0.016
na	My	<i>claytonae</i>	5	1.08 ± 0.056	31.20 ± 4.382	4	0.17 ± 0.009	0.48 ± 0.016
na	My	<i>dimidiata</i>	1	0.97 ± .	28.00 ± .	1	0.17 ± .	0.50 ± .

R: ha = Hawaiian Islands, au = Australia & New Caledonia, na = North America.

G: Dr = *Drosophila*, Hi = *Hirtodrosophila*, Id = *Idiomyia*, Mi = *Microdrosophila*, My = *Mycodrosophila*, Sc = *Scaptodrosophila*, Sz = *Scaptomyza*, Zy = *Zygothrica*.

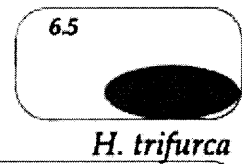
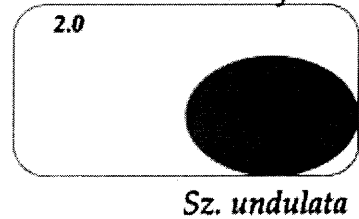
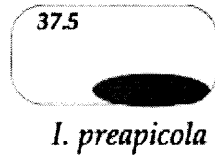
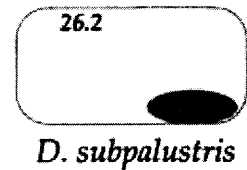
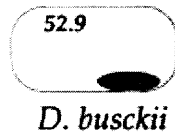
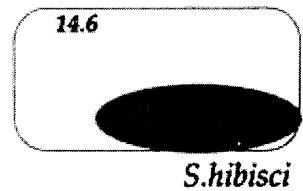
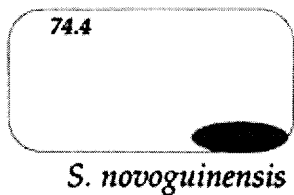
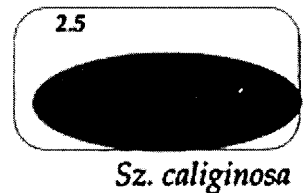
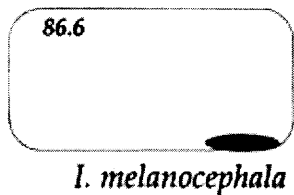
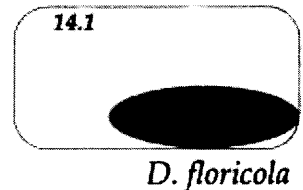
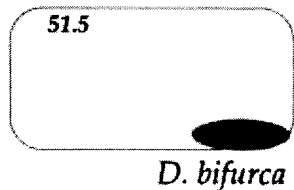
Australasian**Hawaiian****North American****Absolute** **Australasian****Hawaiian****North American****Relative to thorax length**

FIG. 1. Range (low, left to high, right) in egg size for species in the three geographic regions. All figures show egg size as shaded ovals with width < length. The top three comparisons have eggs in open boxes drawn with thorax length as the base and 1/3 thorax length as the height, on an absolute scale. The bottom three comparisons use a relative scale. Mean ovariole number for each species is displayed in the upper left for each case.

TABLE 2. Mean and Coefficient of Variation (CV) for Thorax Length (tl, mm), Ovariole Number and Egg Volume (ev, mm³) of Species in the Three Geographic Regions

Region	n	tl	(CV)	ova	(CV)	ev	(CV)
Australia	29	1.04 ^b	(17.20)	32.69 ^a	(50.46)	0.0089 ^b	(73.48)
Hawaii	36	2.17 ^a	(32.33)	32.95 ^a	(74.07)	0.0492 ^a	(119.45)
North American	36	1.18 ^b	(15.05)	37.22 ^a	(26.28)	0.0074 ^b	(22.01)
ANOVA Region (F _{2,98})		66.3***		0.69		15.75***	
Genus							
Drosophila	39	1.16 ^a	(16.55)	35.44 ^a	(29.86)	0.0076 ^a	(24.85)
Hirtodrosophila	6	1.16 ^a	(6.67)	28.61 ^{a,b}	(48.30)	0.0135 ^a	(93.54)
Idiomyia	29	2.37 ^b	(24.30)	39.98 ^a	(54.84)	0.0386 ^a	(111.79)
Microdrosophila	1	0.81 ^a		22.25 ^{a,b}		0.0044 ^a	
Mycodrosophila	6	0.98 ^a	(14.36)	26.81 ^{a,b}	(12.17)	0.0063 ^a	(30.64)
Scaptodrosophila	12	1.05 ^a	(18.86)	43.62 ^a	(44.96)	0.0079 ^a	(46.84)
Scaptomyza	7	1.35 ^a	(44.15)	3.81 ^b	(30.90)	0.0930 ^b	(100.01)
Zygothrica	1	1.04 ^a		27.50 ^{a,b}		0.0091 ^a	
ANOVA Genus (F _{7,93})		31.67***		5.35***		7.09***	

*** P < 0.001.

^{a,b} represent statistically similar groups for each variable.

The range in ovariole number reflects the egg size variation for each region. The Hawaiian flies range from 2 to 101 ovarioles. The Australasian species had a low of 6.5 and a high of 71.3 ovarioles. These two ranges are much larger than for the North American species (range: 14.1 to 65.4).

Reproductive Effort

Table 3 reports the analysis of reproductive effort considering the following two assumptions: 1) *The scaling relationship with thorax length is constant (tl³)*. In this case change in volume of reproductive tissue is relative to the volume of the female. Values of **rev** × **ova** of Table 3 differ at all levels of the analysis; regions are different, genera are different, species are different, and populations are different. 2) *The scaling relationship with thorax length is constant but not necessarily tl³*. In this case the scaling relationship is estimated for each comparison. Values of **ev** × **ova** of Table 3 are the same in each region and for different populations of *S. hibisci* but differ for genera and the closely related *Scaptodrosophila* species. Comparison of **rev** × **ova** and **ev** × **ova** shows that the rank order of these measures of reproductive effort is the same for regions and species, but with a major shift in

TABLE 3. Analysis of Reproductive Effort for Regions, Genera, 2 Species within Australia and Sites for *S. hibisci*: **rev.ova**, **ev.ova**, **rev** and **ev** Were Evaluated Using Equation 1 and Models 2, 3 and 4. Anti-logarithms of Least-squares Means from the ANOVAs Are Given. If Slopes Were Homogenous the Covariate*Effect Interaction Was Not Included in the Model

Dependent Covariate		Model			
		1 rev.ova	2 ev.ova tl	3 rev ova	4 ev ova, tl
REGION	(species)				
Australian	29	0.219	0.398	0.0140	0.0129
Hawaiian	36	0.098	0.334	0.0058	0.0137
North American	36	0.169	0.356	0.0112	0.0116
ANOVA					
Region F		29.78***	2.12	51.96***	2.72
(dfn, dfd)		(2, 98)	(2, 97)	(2, 97)	(2, 96)
Homogeneity of Slopes (P)			0.12	0.22	0.26, 0.08
GENUS	(species)				
<i>Drosophila</i>	39	0.172	0.344	0.0113	0.0117
<i>Hirtodrosophila</i>	6	0.169	0.349	0.0110	0.0124
<i>Idiomyia</i>	29	0.090	0.384	0.0056	0.0140
<i>Microdrosophila</i>	1	0.188	0.256	0.0123	0.0089
<i>Mycodrosophila</i>	6	0.179	0.300	0.0118	0.0104
<i>Scaptodrosophila</i>	12	0.268	0.467	0.0178	0.0155
<i>Scaptomyza</i>	7	0.132	0.272	0.0063	0.0120
<i>Zygothrica</i>	1	0.225	0.417	0.0151	0.0144
ANOVA					
Genus F		11.94***	4.74***	17.22***	3.14**
(dfn, dfd)		(7, 95)	(7, 93)	(7, 93)	(7, 92)
Homogeneity of Slopes (P)			0.32	0.92	0.58, 0.09
SPECIES	(sites)				
<i>S. acclinata</i>	15	0.382	0.211	0.0554	0.0120
<i>S. hibisci</i>	34	0.256	0.174	0.0367	0.0150
ANOVA					
Species F		49.72***	1.85**	48.91***	4.19*
(dfn, dfd)		(1, 47)	(1, 46)	(1, 46)	(1, 45)
Homogeneity of Slopes (P)			0.03*	0.51	0.63, 0.82
SITES (<i>S. hibisci</i> isolines)					
BCK	9	0.318	0.256	0.0163	0.0131
BEL	9	0.371	0.274	0.0197	0.0134
TRD	5	0.350	0.276	0.0177	0.0136
ANOVA					
Sites F		4.71*	1.02	5.06*	0.63
(dfn, dfd)		(1, 20)	(2, 19)	(2, 19)	(1, 18)
Homogeneity of Slopes (P)			0.22	0.89	0.27, 0.29

* P < 0.05, ** P < 0.01, *** P < 0.001.

TABLE 4. Analysis of Reproductive Effort at One Site (Bellingen, N.S.W.) over Two Weeks in Each of Two Years: *rev.ova*, *ev.ova*, *rev* and *ev* Were Evaluated Using Equation 1 and Models 2, 3 and 4. Anti-Logarithms of Least-Squares Means from the ANOVAs Are Given

Dependent Covariate	Model				
	1 <i>rev.ova</i>	2 <i>ev.ova</i> tl	3 <i>rev</i> <i>ova</i>	4 <i>ev</i> <i>ova, tl</i>	
Year: Week/Strain (females)					
1996:					
Oct. 10/Field	14	0.389	0.287	0.0444	0.0200
Lab.18	2	0.281	0.207	0.0322	0.0111
Lab.21.5	4	0.340	0.230	0.0392	0.0132
Lab.25	3	0.316	0.225	0.0357	0.0132
Oct. 17/Field	36	0.375	0.281	0.0424	0.0181
Lab.18	8	0.323	0.247	0.0361	0.0138
Lab.21.5	11	0.283	0.221	0.0320	0.0130
Lab.25	8	0.301	0.232	0.0342	0.0134
1997:					
Oct. 22/Field	13	0.433	0.299	0.0495	0.0176
Lab.25	3	0.381	0.254	0.0445	0.0167
Lab.29	16	0.391	0.239	0.0445	0.0144
Nov. 1/Field	30	0.573	0.383	0.0654	0.0226
Lab.18	17	0.358	0.262	0.0403	0.0155
Lab.25	12	0.349	0.239	0.0393	0.0140
Lab.29	23	0.343	0.224	0.0382	0.0135
ANOVA					
Year	F	206.7***	20.23*	56.80*	65.72*
	(dfn, dfd)	(1, 2)	(1, 2)	(1, 2)	(1, 2)
Week/Year	F	0.01	0.02	0.03	0.01
	(dfn, dfd)	(2, 11)	(2, 11)	(2, 11)	(2, 11)
Strain/Week/Year	F	12.90***	12.54***	11.30***	25.5***
	(dfn, dfd)	(11, 185)	(11, 184)	(11, 184)	(11, 183)

*P < 0.05, **P < 0.01, ***P < 0.001.

the generic order for *Idiomyia* which moves from the lowest effort to the third highest. A similar analysis for field versus laboratory females collected at the same site during two weeks of two years (Table 4) shows the reproductive effort was different in the two years, similar within years and higher in field collected females as compared to laboratory reared females.

Variance components analysis of the isofemale line data showed no heritability in **rev** \times **ova**. Site variance (0.00090) was eleven times larger than line within site variance (0.00008), while individuals within lines (error) showed the largest contribution (0.00832) to the variance in **rev** \times **ova**.

Egg Size

Egg volume was compared by scaling egg volume with constant thorax length cubed and correcting for the trade-off with ovariole number (Model 3). This analysis shows that regional, generic, species and population categories all differ. On relaxing the assumption that the scaling with **tl** is **tl**³ (Model 4), the analysis indicates that the regional relative egg volumes are similar and populations of *S. hibisci* are also similar. However, generic and sister species egg volumes are still significantly different (Table 3).

The corresponding egg volume analysis for field versus laboratory females of *S. hibisci* (Table 4) shows that volumes differed from year to year and that laboratory females had smaller eggs than the corresponding field collected females.

Allometry of Egg Size and Egg Number

Models 3 and 4 relate egg size to ovariole number with constant female volume set at **tl**³ in Model 3 or estimated as a model component in Model 4. Two comparisons can be made for β_0 . The first is whether the trade-off is equivalent for the groups under comparison ($H_0: \beta_{0a} = \beta_{0b} = \beta_{0c} \dots$) and the second is whether the trade-off is isometric ($H_0: \beta_0 = -1$). The former comparison is a test of homogeneity of slopes in the Analysis of Covariance (Table 3). In all cases the slopes were homogenous, indicating that the trade-off within regional, family, generic, specific and intraspecific levels was similar for each comparison. Regression analysis for all species indicates the trade-off is isometric ($\hat{\beta}_0 = -0.996 \pm 0.069$, $n = 101$, $r^2 = 0.675$) when **tl**³ was used to scale body size. Similar analysis estimating both β_0 and β_1 (Model 4) show the trade-off to be slightly higher than -1 ($\hat{\beta}_0 = -0.869 \pm 0.034$, $\hat{\beta}_1 = 1.847 \pm 0.062$, $n = 101$, $r^2 = 0.928$). The estimates for β_0 and β_1 are given for each category in Table 5.

Analysis of species (*S. hibisci* and *S. acclinata*) over all sites showed that the two species have poor fits to either Model 3 or 4 ($r^2 = 0.203$ and 0.220 , respectively) with β_0 estimates between 0 and -1 ($\hat{\beta}_0 = -0.484 \pm 0.203$ and $\hat{\beta}_0 = -0.207 \pm 0.093$, respectively). Analysis of populations (isofemale line

TABLE 5. Estimates of β_0 (se) β_1 (se) and r^2 for Geographic, Generic and Species Categories. The Three Rows for each Category Represents Models (3), (2) and (4), Respectively

REGION	n	β_0	(se)	β_1	(se)	r^2
Australian	29	-0.895	(0.118)			0.681
				2.067	(0.316)	0.614
		-0.789	(0.103)	1.881	(0.312)	0.743
Hawaiian	36	-1.152	(0.009)			0.894
				2.092	(0.127)	0.889
		-2.012	(0.144)	1.860	(0.163)	0.870
North American	36	-1.107	(0.180)			0.526
				1.454	(0.202)	0.604
		-0.603	(0.107)	1.098	(0.197)	0.560
Phylogenetic NA	32	-0.724	(0.172)			0.363
				1.824	(0.313)	0.522
		-0.506	(0.131)	1.506	(0.276)	0.539
GENERA (Family)	7	-0.804	(0.159)			0.836
				1.919	(0.218)	0.939
		-0.870	(0.110)	2.208	(0.302)	0.967
<i>Drosophila</i>	39	-1.175	(0.148)			0.631
				1.626	(0.183)	0.681
		-0.645	(0.102)	1.229	(0.197)	0.583
<i>Hirtodrosophila</i>	6	-0.926	(0.172)			0.878
				4.730	(1.717)	0.655
		-0.966	(0.188)	4.630	(2.050)	0.901
<i>Idiomyia</i>	29	-1.131	(0.122)			0.760
				1.879	(0.201)	0.764
		-0.905	(0.095)	1.780	(0.224)	0.807
<i>Mycodrosophila</i>	6	-1.913	(0.832)			0.569
				2.335	(0.682)	0.745
		-1.645	(1.227)	2.665	(0.982)	0.718
<i>Scaptodrosophila</i>	12	-1.140	(0.186)			0.789
				1.460	(0.292)	0.714
		-0.897	(0.110)	1.328	(0.325)	0.882
<i>Scaptomyza</i>	7	-0.941	(0.674)			0.280
				1.925	(0.247)	0.924
		-0.927	(0.343)	1.925	(0.275)	0.933
SPECIES (Australia n = sites)						
<i>S. acinata</i>	18	-0.646	(0.271)			0.262
				5.355	(1.009)	0.690
		0.089	(1.759)	0.902	(1.759)	0.117
<i>S. hibisci</i>	34	-0.465	(0.168)			0.194
				1.000	(0.871)	0.040
		-0.124	(0.099)	-0.940	(0.282)	0.236
SITES (<i>S. hibisci</i> n = isolines)						
BCK	9	-0.904	(0.240)			0.669
				2.143	(0.833)	0.486
		0.402	(0.169)	-1.238	(0.488)	0.521
BEL	9	-1.227	(0.583)			0.388
				3.600	(1.374)	0.530
		0.009	(0.438)	1.042	(0.438)	0.151
TRD	5	-0.893	(0.745)			0.324
				0.436	(1.028)	0.058
		-0.263	(0.328)	-0.757	(0.917)	0.661

* P < 0.05, ** P < 0.01, *** P < 0.001.

means of *S. hibisci*) supported an isometric relationship for Model 3 ($\hat{\beta}_0 = -1.01 \pm 0.271$, $r^2 = 0.40$) but did not have significant effects for **tl** or **ova** in Model 4.

Quantitative corrections for phylogenetic effects were only possible for the North American *Drosophila*. The parameter estimates for Model 2 (β_1), Model 3 (β_0) and Model 4 (β_0, β_1) are given in Table 5. Comparison with the comparable results for the North American region without correction shows no significant differences.

DISCUSSION

The analysis of reproductive effort and the trade-off between egg size and egg number is premised on the following ecological, physiological and evolutionary assumptions:

- 1. Egg volume is independent of female age, diet, temperature and humidity.** This assumption has not been investigated to any great extent in Drosophilidae (but see Avelar, 1993). Bernardo (1996) discusses the general expectation that propagule size should be affected by maternal condition and her ecological situation. This expectation is supported by analysis comparing the size of eggs from field caught *S. hibisci* females with those of females from the same site following one generation of laboratory rearing from larvae to adult stage (Table 4). Field-caught females had larger eggs (23–60% larger) than laboratory-reared flies, indicating that age (relatively young in the laboratory), climatic differences, or nutrition (unknown in the field) could be important to egg size.
- 2. Oviposition opportunities are unlimited.** This assumption also is likely false. Both seasonal and species differences in reliability of oviposition substrate may compromise nearly all levels of comparative analyses. For instance, a prolonged break in oviposition opportunity can cause ovariole activity to cease as mature eggs accumulate in the abdomen (King and Sang, 1959). With respect to species differences, rare oviposition opportunity coupled with unconstrained larval resources when the opportunity is present is believed to select for a reproductive strategy featuring many ovarioles producing small eggs (Montague et al., 1981, Kambysellis et al., 1995). Without knowledge of how body size covaries with substrate availability, it is not possible to know how violation of this assumption influences the egg size/egg number trade-off.

3. Egg number is equivalent to ovariole number. We expect this assumption often to be false. Some ovarioles may not be functional and the proportion of nonfunctional ovarioles may increase with female age (Gasser et al., 2000). Our experience with *S. hibisci* is that females rarely have egg numbers equivalent to ovariole numbers in either field or laboratory reared flies. In many cases, only one or very few ovarioles are active. This situation appears to be true also for Hawaiian flower-breeders (Montague, 1984, 1989) and leaf-breeders (Kambysellis et al., 1995). On the other hand, Hawaiian bark-breeders typically have many ovarioles with multiple eggs developing in each ovariole (Kambysellis et al., 1995). Consequently, ovariole number is better viewed as an index of potential instantaneous egg production for each species. Consistent with this viewpoint, David (1970) and Boulétreau-Merle et al. (1982) observed a positive correlation between ovariole number and the maximum daily rate of oviposition (but see Wayne et al., 1997).

It is also worth noting here that detailed studies of the function of male accessory gland proteins following insemination in *D. melanogaster* have determined that seminal fluid (i.e., the “sex peptide” 26Aa) can enhance the egg production rate of females (Chen et al., 1988; Kalb et al., 1993; Herndon and Wolfner, 1995). This effect is believed to have arisen through sexual conflict over sperm use (Eberhard, 1996; Holland and Rice, 1998). Variation among species or populations in the intensity of postcopulatory sexual selection and in the outcome of sexually antagonistic coevolution (Rice, 1998) could therefore contribute to substantive differences in intrinsic and realized egg production rates.

4. Instantaneous reproductive effort is a good index of total reproductive effort. Even if oviposition opportunities are unlimited, females of different species, populations, or samples may not produce per unit volume of egg at the same rate or cost. We examine the egg size-egg number relationship using a static measure of reproduction (i.e., egg size \times ovariole number). At best, this variable reliably indicates the instantaneous potential for reproduction. However, an extension to lifetime reproductive effort would require knowledge about the dynamics of egg production and oviposition in relation to oviposition opportunity and longevity of the female. Considering such extensions, the reproductive effort and the parameter α may not be constant for females of different sizes, different senescence patterns (Carlson et al., 1998) or in different ecological situations (Kambysellis et al., 1979). Moreover, true reproductive effort, which is the proportion of energy flowing through

TABLE 6. Goodness of fit Model 2 at Each Level of the Taxonomic Organization

	n	r ²	$\beta_1 \pm se$
Genera	7	0.94	1.92 \pm 0.22
<i>Scaptodrosophila</i>	12	0.71	1.46 \pm 0.29
<i>S. hibisci</i>	34	0.04	1.00 \pm 0.87
Populations (<i>S. hibisci</i>)	23	0.54	1.64 \pm 0.30

the organism that is devoted to reproduction, cannot be determined confidently by any static measure (Hirshfield and Tinkle, 1975).

5. **There is a consistent relationship between body size and reproductive effort.** If species differ in their reproductive effort, or in the proportion of their reproductive effort devoted to egg production, then the trade-off between egg size and number may not be discernable. For example, an increase in egg size with no reduction in egg number may not indicate a lack of trade-off between these characters if it is also associated with an increase in reproductive effort. Unfortunately, no criterion is known for quantifying investment in egg production that is independent of egg size and number.
6. **Allocation to reproduction is independent of the trade-off between egg size and egg number.** Most theoretical treatments of egg size evolution have assumed that egg size and total resources devoted to reproduction are optimized independently (Vance, 1973a, 1973b; Smith and Fretwell, 1974; Brockelman, 1975; Lloyd, 1987; McGinley et al., 1987; Sargent et al., 1987; Sinervo et al., 1992; but see Winkler and Wallin, 1987). Once total reproductive allocation is optimized, resources are divided among the maximum number of optimally sized offspring, resulting in a trade-off between offspring size and number. This assumption has been challenged empirically by a recent study by Schwarzkopf et al. (1999) in which selection for increased and decreased egg size was imposed on *D. melanogaster*. Total reproductive allocation did not change in lines selected for large eggs but was reduced in lines selected for small eggs.
7. **Body weight is proportional to the cube of a linear dimension, i.e., thorax length cubed.** This assumption has not been tested for many *Drosophila* species because most studies report the correlation between body weight and thorax length and not with thorax length cubed. We reanalyzed data for *D. hydei* reported by Pitnick and

Markow (1994) and found the regression of log (dry weight) on log (thorax length) had a coefficient less than the expected value of 3 ($\hat{\beta} = 2.47 \pm 0.127$, $n = 20$, $r^2 = 0.954$). However, Robertson and Reeve (1953) argued that the relationship of weight and thorax length cannot be expected to be exact because weight is more variable than thorax length. Their paper reports that females can increase weight by 40% and males 5% during adult life. As a consequence, thorax length is considered to be a more accurate measure of size. Robertson and Reeve (1953) also discuss the functional relationship (Kermack and Haldane, 1951) and the expectation that the regression of log (weight) on log (thorax length) should have a slope of 3. However, Robertson and Reeve estimate the slope to be 1.64 in experiments with males of *D. melanogaster*.

Despite these considerations, our use of thorax length cubed to correct for size differences in expression (1) and Model 3 is a geometric scale correction (volume/volume) and does not take into account differences in specific gravity or the state of tissues (i.e., a mass consideration).

- 8. Field caught and laboratory reared females represent the same sample.** Here we compare the egg size/number trade-off between different continental radiations. However, North American flies examined were primarily obtained from laboratory cultures whereas the Hawaiian and Australasian flies were mostly collected from the field. Intraspecific comparison of field-caught and laboratory reared females of *S. hibisci* (Table 4), however, shows the trade-off is essentially the same for both samples even though reproductive effort is generally higher for field-caught individuals (a similar reduction in reproductive effort in the laboratory versus the field has been described for a grasshopper; Kriegsbaum, 1988 as cited in Stearns, 1992). The primary reason for this difference is that egg volume is always larger in field-caught flies than in laboratory reared ones ($F_{3,186} = 75.6$, $P < 0.001$).
- 9. Taxa represent independent data for each comparison.** It is recognized that related taxa do not constitute independent data points for statistical purposes (Felsenstein, 1985; Harvey and Pagel, 1991). Nevertheless, because detailed phylogenetic relationships are unknown for the Hawaiian and Australasian taxa, only data for the North American taxa could be properly analyzed using phylogenetically independent contrasts (Harvey and Pagel, 1991). This analysis showed that phylogenetic corrections resulted in similar parameter estimates (Table 5). Thus, for the North American *Drosophila* species the relationships between egg size, ovariole

number and body size have not been subject to significant phylogenetic constraint. Furthermore, the lack of statistical difference between slopes generated by interspecific regression and those generated by intergeneric regression (Figs. 2 and 4) suggest that these data are robust to violations of this assumption.

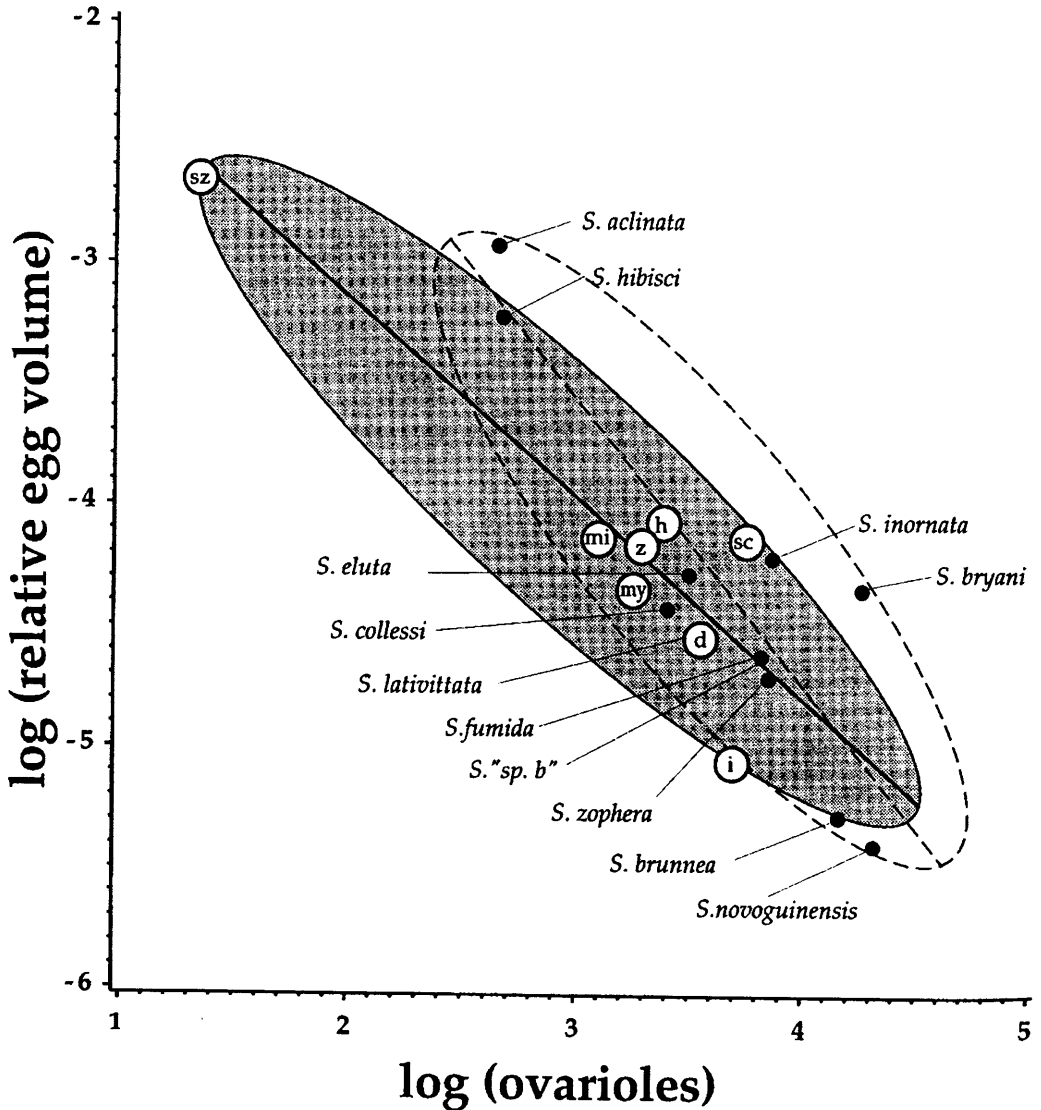


FIG. 2. The trade-off between relative egg size and ovariole number for genera of the Family (circles with generic abbreviations) and for species of *Scaptodrosophila* (solid circles). The range of the family regression line and the variation among genera is shown as a solid line and shaded oval. The genus line and variation is shown as a dashed lined and open dashed oval. sz = *Scaptomyza*, h = *Hirtodrosophila*, sc = *Scaptodrosophila*, z = *Zygothrica*, mi = *Microdrosophila*, my = *Mycodrosophila*, d = *Drosophila*, and i = *Idiomyia*.

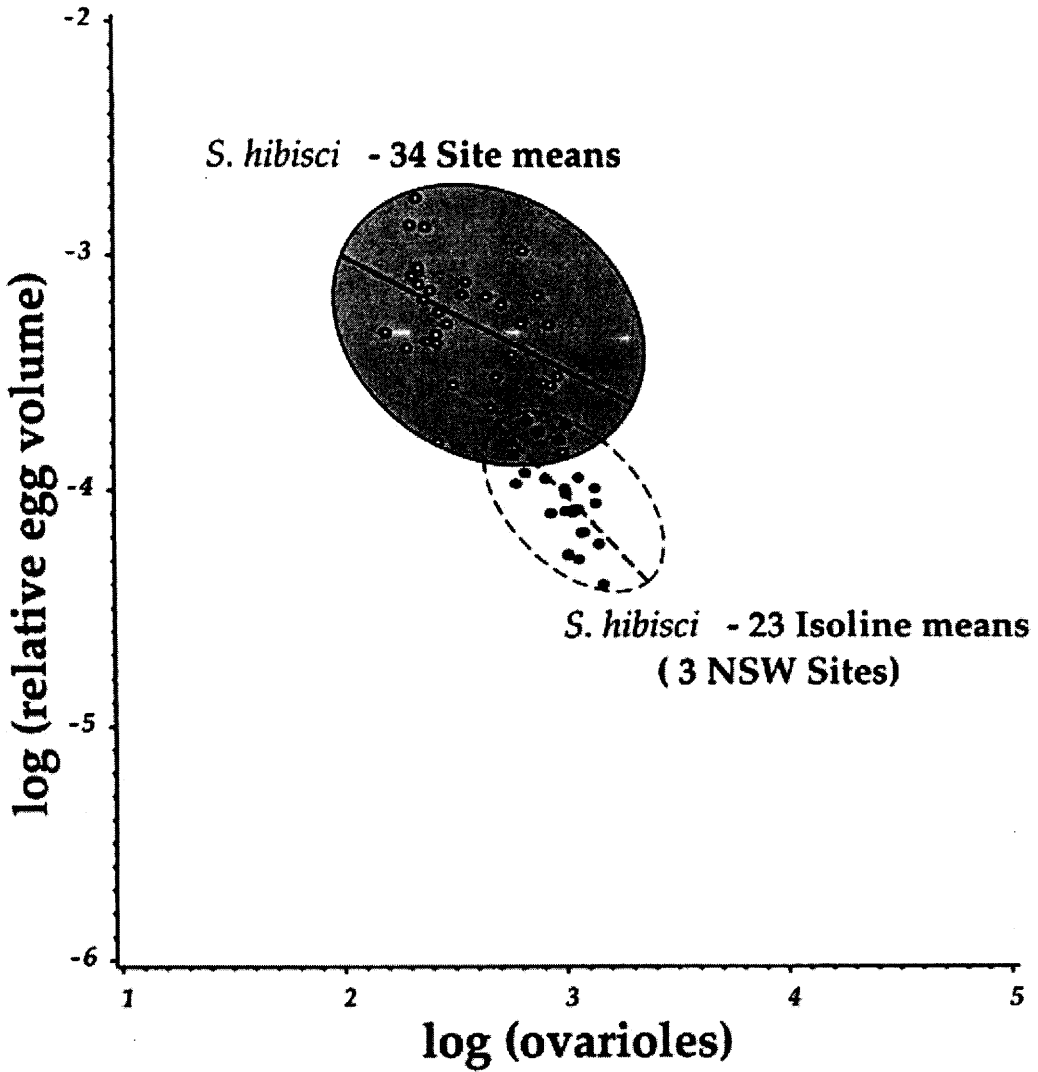


FIG. 3. The trade-off between relative egg size and ovariole number for population means (sites) of *S. hibisci* in eastern Australia (open circles with shaded oval boundary) and for isoline means of three sites in N. S. W. (closed circles with dashed line and dashed oval boundary).

General

The very broad conclusion of the interspecific comparisons is that the relationship between egg size and ovariole number is close to the isometric value, $\beta_0 = -1$, across geographic regions and taxonomic categories (Table 5). However, the $\beta_0 = -1$ slope was not always mirrored in the spatial and temporal analysis of *S. hibisci*, where $\beta_0 > -1$ for the species comparison.

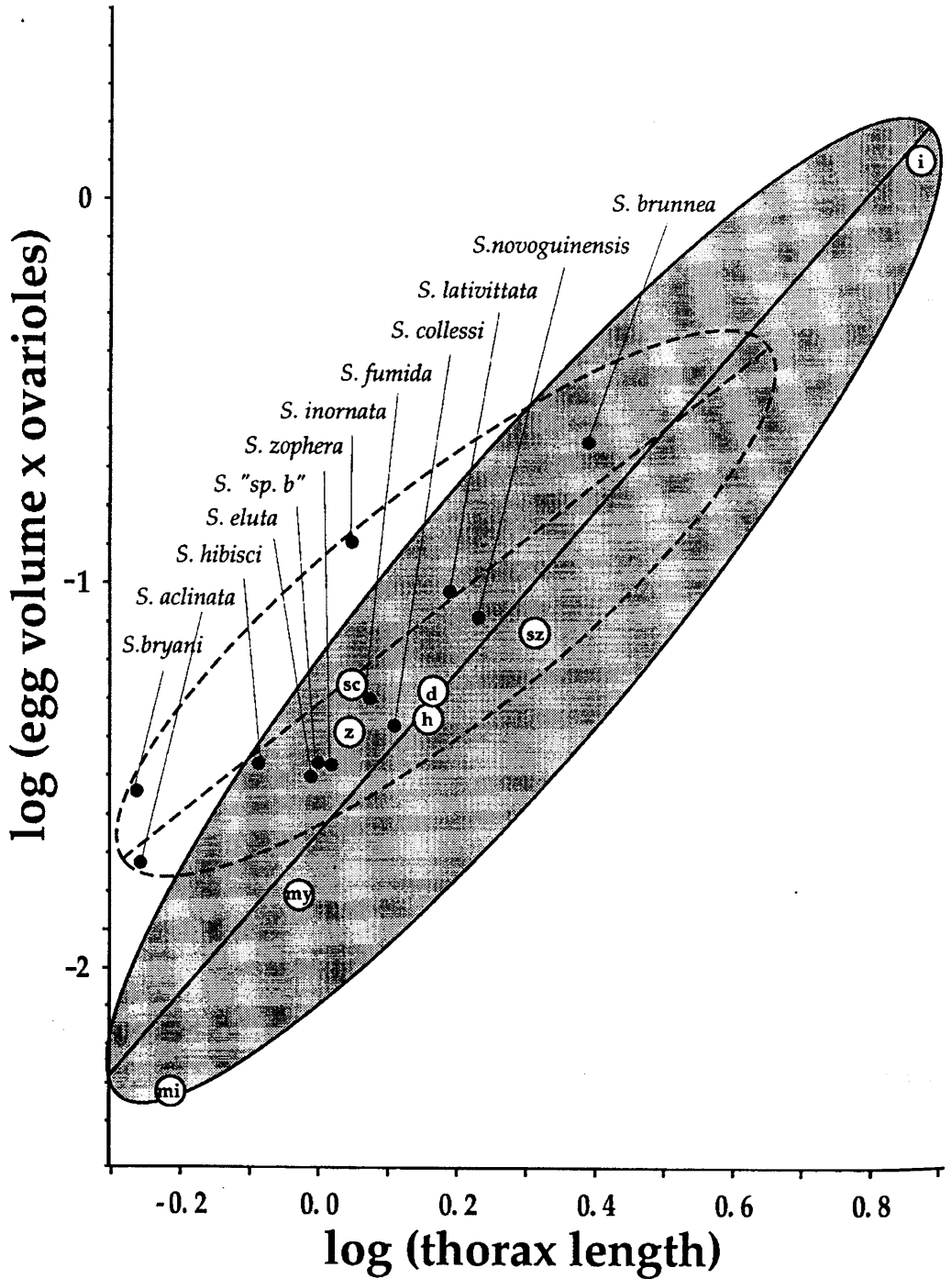


FIG. 4. Reproductive effort as a function of thorax length for genera (circles with generic abbreviations) and for species of *Scaptodrosophila* (solid circles). The extent of the family line and variation among genera is depicted as a solid line enclosed in a shaded oval. The extent of the within genus line and variation is shown as a dashed line within a dashed open oval. sz = *Scaptomyza*, h = *Hirtodrosophila*, sc = *Scaptodrosophila*, z = *Zygothrica*, mi = *Microdrosophila*, my = *Mycodrosophila*, d = *Drosophila*, and i = *Idiomyia*.

The relationship between the inter- and intraspecific pattern is of interest because the microevolutionary process is expected to translate into macro-evolutionary patterns (Hansen and Martins, 1996). A comparison of β_0 for *S. aclinata* and *S. hibisci* with the β_0 estimated from *Scaptodrosophila* species means shows that even though the intraspecific slopes are generally > -1 , their 95% confidence intervals overlap with the confidence interval of the interspecific estimates of the parameter. Figures 2 and 3 compare four levels in the taxonomic hierarchy (Family > Genus > Species > Population) for the trade-off between relative egg size and ovariole number. The increased variation and general lack of fit to Model 3 is apparent at the species level but not above or below.

One problem with the comparison of spatial and temporal variation of β_0 for *S. hibisci* to higher levels of divergence is that this species is on the upper extreme of egg size for the *Scaptodrosophila* species we examined (Fig. 2). Thus, *S. hibisci* could represent a species under pressure to maintain a large egg of constant size, resulting in $\beta_0 > -1$. Unfortunately, no other *Scaptodrosophila* species had an adequate sample size to make a meaningful comparison at intermediate points on the reproductive continuum. Eleven of the North American species had sample sizes of eight or more. These species were used to compare the CV for egg volume with mean thorax length and mean ovariole number. The expected quadratic relationship of reduced variation in egg size at the extremes was not supported for either comparison. This result is not consistent with the notion that variation in egg size is constrained at extremes of the distribution. A similar analysis with the CVs for egg length and egg width of the Hawaiian species resulted in the same conclusion.

Allometry of Reproductive Allocation ($ev \times ova$) and Thorax Length

Expression (1) estimates reproductive effort as the ratio of two volumes and assumes thorax length cubed approximates fly volume. Under this assumption changes in reproductive effort should reflect changes in allocation to locomotion, maintenance, longevity or other non-reproductive activities of the female. Model 2 does not make this assumption but estimates the scaling relationship (β_1) that would maintain a constant reproductive effort.

Table 3 indicates that the slopes of the regression of $\log(ev \times ova)$ on $\log(tl)$ are homogenous for all levels of comparison except for the two species (*S. aclinata* versus *S. hibisci*). In general, the slopes were closer to 2 than to 3 (Table 5). The goodness of fit (captured in r^2 , Table 5) was poor

for *S. hibisci* but fairly good at higher and lower levels of taxonomic organization.

Model 4 evaluates egg size as a function of both egg number (**ova**) and fly size (**tl**). The β_1 estimates were homogeneous for each level of comparison (Table 3). Table 5 shows estimates of β_1 from Model 2 and Model 4 were not significantly different for regional, intergeneric (family) and generic comparisons in each analysis. In addition, improvement in fit from the two parameter models (2 and 3) to the three parameter model (4) was generally non-significant and thus the two parameter models (2 and 3) are adequate to estimate the relationships. The species data showed poor fits to Model 4 and the isofemale line analysis was mixed (Table 5).

Evolution of Reproductive Effort

The general conclusion of the reproductive effort analysis is that bigger females allocate proportionally less of their resources into reproductive tissue. There are several possible explanations for this result.

Ecological: If oviposition opportunities are rare with relatively long spans of waiting, then selection will favor greater longevity. This can be achieved in a number of ways, one of which is to favor larger animals with greater investment into maintenance at the expense of reproductive tissue. Kambyssellis et al. (1995) describe this reproductive strategy for bark-breeding Hawaiian picture-winged species (*Idiomyia*). These species have rare oviposition opportunities and large bodies. They have many ovarioles and make many eggs per ovariole for each oviposition opportunity. These species are at the low end of the reproductive continuum with relatively low reproductive effort relative to body size. They also occupy the "many small eggs" corner of the trade-off expectation (Fig. 1). Small eggs may mean that the habitat imposes little pressure on larval development time. Bark-, flux- and large fruit-breeding habitats fit this criterion. The larval habitats of most of the *Scaptodrosophila* are not well known but the reproductive effort of *S. brunnea* [found on the flux of *Frareiseedendron laurifolium* (Sterculiaceae)] is consistent with the Hawaiian model.

Furthermore, when Schwarzkopf et al. (1999) applied selection for small eggs in *D. melanogaster*, the reproductive allocation decreased. This result parallels our finding that large flies with many small eggs exhibit reduced reproductive effort. However, the assumption that each ovariole has one egg is important to consider, especially if there are deviations from this pattern as mentioned above. Kambyssellis and Heed (1971) report the number of eggs per ovariole in field caught females in the Hawaiian species. Two statistics are derivable from their data: 1) a positive correlation between ovariole number and eggs per ovariole ($r = 0.710, n = 36, P < 0.001$),

and 2) the estimate of β_1 in Model 3 is close to the cube rather than the square ($\hat{\beta}_1 = 3.259 \pm 0.315$, $r^2 = 0.759$). Thus, when the positive relationship between egg number per ovariole and ovariole number is considered, reproductive effort is close to constant in the Hawaiian taxa. This type of analysis was not possible for the regions because either the flies assayed were from laboratory cultures or the necessary observations on eggs per ovariole were not made.

If oviposition opportunity is common but the larval habitat constrains the juvenile stage (e.g., poor nutrients or a time limit on nutrient availability) then well provisioned eggs that enhance larval competitive ability or growth rate should be favored. This condition may require more reproductive effort and, therefore, less material devoted to maintenance of the adult. The Hawaiian taxa that live as larvae under these conditions are the leaf-breeders and flower-breeders. These flies are on the higher end of the reproductive effort continuum and also occupy the "few-large eggs" corner of the trade-off expectation. It is noteworthy that all three geographic regions have taxa that exemplify this strategy (few-large eggs) and in all cases they are flower-breeders.

Morphological: The $\beta_1 \cong 2$ result (i. e., the reproductive effort scales to the square of thorax length and not to the cube) may be explained by the fact that ovarioles and eggs are in the expandable abdomen rather than a fixed space such as the thorax. It is thus possible that increasing a linear dimension (thorax length) increases the capacity for expansion of the abdomen such that it does not require as large an increase in egg size \times egg number to achieve the same proportional reproductive effort. However, even after accounting for this (Model 2 and 4), residual differences in reproductive effort still existed for different genera of the family and for the two closely related species of *Scaptodrosophila* (Table 3). In this case, the size factor was not important. Genera with larger flies exhibited relatively high and low effort (*Idiomyia* and *Scaptomyza*, respectively), as did genera with smaller flies (*Scaptodrosophila* and *Mycodrosophila* had relatively high and low effort, respectively) (Fig. 4). The only significant correlation with residual reproductive effort was with mean ovariole number.

In addition, there may be a physical space trade-off in the abdomen if crop volume (which can ultimately go to either maintenance or reproduction) competes with ovarian volume. Even though the crop is anterior to the ovaries, when either increases in volume, space for the other may become restricted.

The linear relationship between log of reproductive volume and log thorax length is consistent in the phylogenetic hierarchy (Family > Genera > Species > Population > Individual). The range covered by the relationship is decreasing until the level of population, i. e., Family > Genus > Species > Population < Individual. The analysis of the isofemale lines of *S. hibisci* did

not show significant heritability with most of the variance within lines (89%) and little (1%) for lines within populations. This result is illustrated in Figs. 4, 5 and 6. The increased variance from isofemale lines to females (within line) illustrates the large environmental component that influences reproductive effort and the resulting phenotypic plasticity.

The extraordinary range of egg size and ovariole number in the family *Drosophilidae* appears to have occurred independently in several regions

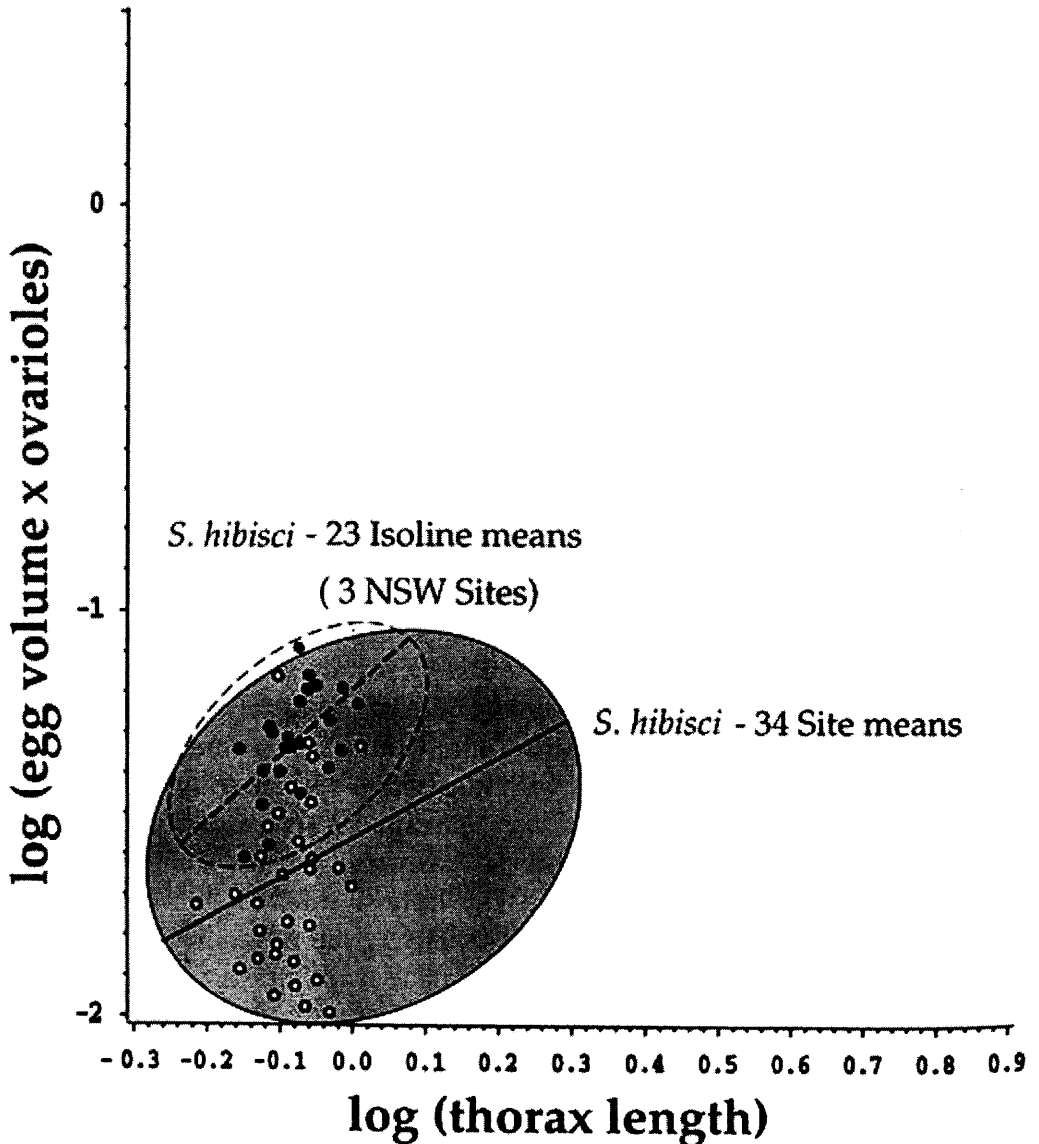


FIG. 5. Reproductive effort as a function of thorax length for population means (sites) of *S. hibisci* in eastern Australia (open circles with shaded oval boundary) and for isofemale means of three sites in N. S. W. (closed circles with dashed line and dashed oval boundary).

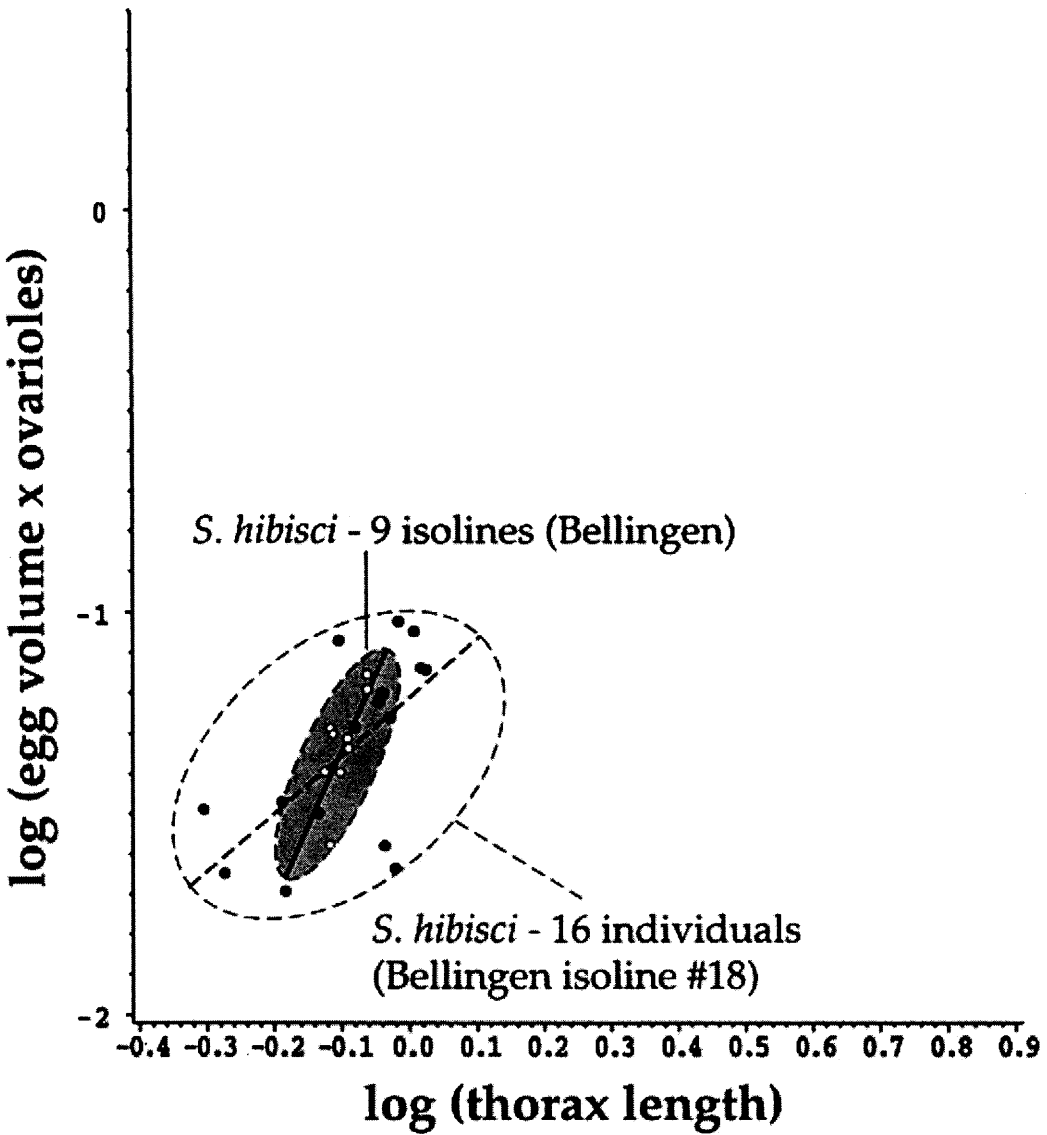


FIG. 6. Reproductive effort as a function of thorax length for isofemale lines of *S. hibisci* collected from Bellinghen, N. S. W. (open circles with shaded oval boundary) and for individuals of a representative isoline (closed circles with dashed line and dashed oval boundary).

of the world. The negative relationship between egg size and number is also common and is manifested at all levels of the taxonomic hierarchy. Even though the relationship is robust to violation of several assumptions, deviations from isometry are worthy of further study. Lifetime fecundity studies, larval habitat discovery and more precise body metrics for relating body mass to reproductive effort would contribute to a better understanding of reproductive trade-offs in these flies.

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