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REPRODUCTIVE CHARACTERISTICS OF THE FLOWER BREEDING *DROSOPHILA HIBISCI* BOCK (DROSOPHILIDAE) IN EASTERN AUSTRALIA: GENETIC AND ENVIRONMENTAL DETERMINANTS OF OVARIOLE NUMBER

WILLIAM T. STARMER,[1,2] MICHAL POLAK,[1] LARRY L. WOLF,[1] AND J. S. F. BARKER[3]
[1]*Biology Department, Syracuse University, Syracuse, New York 13244*
[2]*E-mail: wstarmer@mailbox.syr.edu*
[3]*Department of Animal Science, University of New England, Armidale, New South Wales 2351, Australia*

Abstract.—Quantitative genetic analysis of the ovariole number of the Australian *Hibiscus* flower-breeding *Drosophila hibisci* Bock was conducted on populations from two localities along a latitudinal cline in ovariole number previously observed in the species (Starmer et al., in press). Parental strains, F_1, F_{1r} (reciprocal), F_2, backcross, and backcross reciprocal generations were used in a line-cross (generation means) analysis. This analysis revealed both additive and epistatic effects as important determinants of variation in ovariole number when larvae were reared at 25°C. Maternal effects and maternal-by-progeny genetic interactions were not significant. These results are comparable to previous studies that document epistatic components as genetic determinants of ovariole number in *D. melanogaster*. Parallel studies on ovariole number in *D. hibisci* parental and hybrid generations (F_1 and F_{1r}) reared as larvae at three temperatures (18°, 21.5°, and 25°C) showed environmental effects and genotype-by-environment interactions as significant influences on the phenotype. Maternal effects were present when temperature of larval development was considered and significant, nonlinear environmental effects were detected. Field collections of *D. hibisci* females showed that field conditions result in significant departure of ovariole number from comparable laboratory reared females. The significant epistatic genetic effects, genotype-by-environment interactions, and maternal effects indicate that the genetic architecture of traits, such as ovariole number, may be more complex than often acknowledged and thus may be compatible with Wright's view of a netlike relationship between the genome and complex characters (Wright 1968).

Key words.—*Drosophila hibisci*, ecological genetics, epistasis, genotype-by-environment interactions, *Hibiscus*, latitudinal cline, line-cross analysis, maternal effects, ovariole number.

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In his classic paper on the correlation between relatives, R. A. Fisher (1918) introduced the notion of "dual epistacy" to account for deviations from linearity due to interactions between distinct genes involved in determining quantitative characters. Fisher did not elaborate on the role of epistasis in determining quantitative phenotypes and implied that the second-order contribution of gene-gene interactions supplied only small quantities that could be generally ignored. Sewall Wright (1968), however, recognized the universality of interaction effects (i.e., epistasis) as a generalization of his model of a netlike relationship between the genome and complex characters. The question of the importance of epistasis in the evolution of quantitative characters remains a central problem in understanding adaptation and genetic divergence in natural populations. Other nonlinear effects such as genotype-by-environment interactions in conjunction with geographical population structure and fluctuating population size are also potentially important determinants of quantitative features of organisms. These factors along with epistasis, environmental influences, and the traditional additive genetic contribution provide a fairly complete scheme for understanding and dissecting the causes of phenotypic variability.

The degree of interaction and independence of genetic and environmental determinants of quantitative phenotypes may influence the mode of adaptation and also may constrain or facilitate the types of solutions to ecological problems (Hard et al. 1993). Many ecological-genetic studies identify phenotypes that are relevant to their question (e.g., evolutionary response to predation pressure). They then examine whether phenotypic variation has a genetic basis and how much environmental conditions influence the organism's phenotype (Barker et al. 1990). The potential importance of epistasis and genotype-by-environment interaction generally is recognized to be relevant to ecological-genetic questions, but the difficulty in determining their significance and estimating their magnitude often precludes including them in ecological-genetic studies (Barker 1974, 1979; Hedrick et al. 1978). The search for, and identification of, cases in which epistasis is present has been more common since tests for detecting it were devised (Mather 1949; Hayman 1958, 1960).

Theoretical considerations of the role of epistasis in the evolution of natural populations include the following features. Epistasis (1) can, in conjunction with genetic drift, enhance the rate of genetic diversification (Bryant et al. 1986; Goodnight 1987, 1988, 1995); (2) can mask genotypes and thus hide additive variation, thereby limiting the range of phenotypes exposed to the selective process (Gimelfarb 1989); (3) may be involved in the expression and increase in the additive genetic variation after founding events (Templeton 1980; Goodnight 1988); (4) can be involved in producing heterosis (Falconer 1989; Schell and Cockerham 1992); (5) is important for the operation of interdemic selection because it facilitates the generation of between-deme genetic variation (Wright 1931, 1969, 1978; Wade and McCauley 1984); and (6) is central to Wright's shifting balance theory of evolution, where gene interactions are the foundation for promoting coadaptation of genes (Wright 1935, 1952, 1969; Goodnight 1995).

Genotype-by-environment interactions also can enhance genetic diversification in isolated populations, especially

when the "best" genotype varies among environments (Falconer 1989; Gillespie and Turelli 1989; Pray et al. 1994). Just as epistasis can be the basis for "coadapted gene complexes" within a population, genotype-by-environment interactions can be the foundation for adaptations realized by organisms living in different environments. In addition, the expression of epistasis can depend on the environment (Yermano and Allard 1961; Jinks et al. 1973; Blows and Sokolowski 1995) and the degree of expression of a quantitative trait (Blows and Hoffman 1996).

Populations that are sufficiently separated and experience different environments are suitable for investigation of genetic, environmental, and genotype-by-environment interactions because they are likely to experience selective pressures that lead to genetic divergence. Phenotypic clines are useful in this context because the phenotypes often experience regular changes in environmental variation over short and long distances.

Clines are usually interpreted as resulting from either an evolutionary response to environmental conditions that have persisted over relatively long periods of time or from short-term processes such as recent hybridization and subsequent spread of intermediate phenotypes from the hybrid zone (Endler 1977). Phenotypic clines can also result from constant genotypes along the cline when differential phenotypic expression is a consequence of short-term ecological conditions. We have been investigating a latitudinal cline in the number of ovarioles carried by females of the *Hibiscus* flower-breeding *Drosophila hibisci* Bock. *Drosophila hibisci* uses *Hibiscus* section *Furcaria* DC (Malvaceae) flowers at all stages of its life cycle (Cook et al. 1977). The plants grow as small, spatially isolated, normally monospecific stands of usually less than 50 trees, often less than 10 trees. The flowers are used for larval and adult nutrition, as a site for courting and mating (Polak et al., in press) and as shelter. *Hibiscus* flowers used by *D. hibisci* are one-day flowers, opening in the morning and senescing and closing in the evening. These closed blossoms sometimes remain on the plant, but usually detach by the next day and fall to the ground beneath the parent tree or bush. Adult females insert their eggs into the petal tissue inside the open flower. Depending on the temperature, larvae take about a day to hatch. Larvae feed on flower tissues as well as the microbes associated with the decaying blossom. Pupation probably occurs in surrounding debris or in the soil.

A survey of 36 populations ranging from 20.8° to 34.4° South latitude along the east coast of Australia showed that females from northern populations have fewer ovarioles (10–13) than females in southern populations (15–20 ovarioles). Mean ovariole number was not correlated with species of *Hibiscus* used as a larval substrate or with floral or habitat characteristics of the local host *Hibiscus* populations (Starmer et al., in press). These characters included density of trees or bushes, flower weight, petal length, microorganisms present, density of flies, and density of associated beetles. Mean ovariole number of the 36 populations was significantly correlated with mean thorax length, mean local temperature, and mean daily rainfall (5–19 days prior to collection). These results suggest that the larval abiotic environment may influence the reproductive potential of the population by determining the ovariole number of the resulting females, but do not exclude the possibility that the populations have adapted to different predictable larval environments present along the latitudinal gradient.

As a first step in determining what environmental and genetic factors are responsible for the population differentiation of ovariole number, we have experimentally investigated the effect of larval and pupal developmental temperature in conjunction with quantitative genetic analysis between two populations that differ in their mean ovariole number. These experiments were conducted to ascertain (1) the influence of developmental temperature; (2) the magnitude of genetic determination, including maternal effects and digenic epistasis; and (3) the degree of genetic by environment interaction.

Materials and Methods

Flowers used in the laboratory were obtained daily from *H. heterophyllus* plants growing in a greenhouse at the University of New England, Armidale, New South Wales.

Two parental strains of *D. hibisci* were obtained from two sites 1157 km apart. The northern strain, designated "**n**," was derived from adults reared from 131 flowers of *H. heterophyllus* collected in Eungella National Park, Queensland (21.139°S, 148.507°E). The other strain, "**m**," originated from 181 flowers of *H. heterophyllus* collected from the middle of the geographic distribution of *D. hibisci* near Port Macquarie, New South Wales (31.500°S, 152.804°E).

Original parental strains of *D. hibisci* used in self and cross generations were reared in the laboratory at 25°C from eggs deposited in field-collected flowers. Adults were reared by placing individual flowers with eggs into open plastic bags inside glass milk bottles containing dry, autoclaved sand. After pupae started to form, 10 mL of water was added to each bottle to moisten the sand. Emerging adults were aspirated from the bottles each day. Sexes were separated and placed either in sugar vials (5% sucrose, 1.5% agar) or into cages containing flowers inserted into containers of water resting on dry sand. The cages received one to three newly opened flowers every day. Old flowers were left in the cages or removed and placed in bottles at the appropriate temperature for larval development. Cages were used to mature and maintain females as virgins either for crosses or for dissection of their reproductive structures.

Test Lines

The sources for the lines and crosses used in this study are listed in Table 1. Adults reared from the field-collected flowers incubated at 25°C were allowed to mature, then selfed and crossed reciprocally to form four lines (m⊗, n⊗, F_1, F_{1r}). These matings were conducted at 25°C in duplicate cages each with 20 pairs of females and males. Flowers with eggs were collected from these eight cages daily over a 15-day period, put into bottles, split daily into three groups, and placed at three different temperatures (25.0°, 21.5°, and 18°C). Adults of the four lines that were reared at 25°C were used to form five segregating lines composed of the four backcrosses and the F_2 generation as detailed in Table 1 and Figure 1. Each of these lines was obtained by placing 18–20 pairs of males and females in cages at 25°C. After five days,

TABLE 1. Source of genetic lines used in the analyses.

Line	Source
m	Reared at 25°C from field collected flowers, near Port Macquarie
m⊗	Self of m
m●	Pooled m and m⊗ reared at 25°C
n	Reared at 25°C from field collected flowers, Eungella National Park
n⊗	Self of n
n●	Pooled n and n⊗
F_1	n females × m males
F_{1r}	m females × n males
F_1●	Pooled F_1 and F_{1r} used in measurements
F_{1p}	Pooled F_1 and F_{1r} used in crosses
F_1●●	Pooled F_1● and F_{1p}
F_2	Self of F_{1p}
Bcm	F_{1p} females × m⊗ males
Bcmr	m⊗ females × F_{1p} males
Bcn	F_{1p} females × n⊗ males
Bcnr	n⊗ females × F_{1p} males

each reciprocal backcross cage was supplemented with 10–12 pairs of males and females. Flowers containing eggs of these five lines, collected daily over a 10-day period, were placed in bottles and incubated at 25°C.

For females in each test generation, adult thorax length was measured from the anterior end of the thorax to the posterior end of the scutellum, and the abdomen was dissected in Ringer's solution. The ovaries were fixed for about 1 minute in FAA (6:16:1:30, formalin:absolute alcohol:glacial acetic acid:distilled H_2O), and the number of ovarioles in each ovary was counted. Asymmetry in number of ovarioles was calculated as the difference in ovariole number between the two ovaries. If an egg was present in the uterus, its length and width were measured. Egg volume was approximated by calculating the volume of a prolate spheroid as $(1/6)\pi$(egg length)(egg width)^2 (Atkinson 1979; Montague et al. 1981).

Line means and standard errors for ovariole number were estimated by adjusting the means with an analysis of covariance using thorax length as the covariate. The distributions of ovariole number for the parental lines were tested for normality with the Shapiro-Wilk statistic, W. Both distributions fit the normal distribution (**n**: $W = 0.96$, $P = 0.11$; **m**: $W = 0.97$, $P = 0.24$), and neither square-root nor logarithmic transformations of ovariole number improved the fit to the normal. We thus used a linear scale for ovariole number because it conforms with the normality assumption for tests of significance and it permits comparisons with other studies on the genetic determination of ovariole number that used the same scale.

Models

Two main models based on generation means analysis (Mather and Jinks 1982) were used to estimate genetic and environmental contributions to the observed means. The first was a genetic model using the self, F_1, backcross, and F_2 lines reared as larvae at 25°C. This model was used to evaluate two basic modes of genetic activity, one with and one without maternal effects. The model without maternal effects included the following composite genetic effects (additive, d; dominance, h; digenic epistasis, i, j, and l), whereas the maternal model involved gene activity of progeny (additive, d; maternal additive, dm; maternal dominance, hm; and digenic epistasis between maternal and progeny genotypes, d.dm, d.hm). Other digenic epistasic parameters such as progeny dominance by maternal dominance (h.hm) are not possible to estimate for the lines produced in this study because they either cannot be discriminated or their coefficients are linear combinations of the coefficients for other simpler terms in the model. Given that we observed nine means, models with more that eight parameters could not be evaluated. As a consequence, a full digenic epistatic model with all parameters of progeny, maternal and maternal by progeny interaction was not possible to evaluate for the lines raised at 25°C.

The second model was used to estimate the contribution of additive, dominance, and maternal effects in addition to environmental effects of larval developmental temperature. The self and F_1 lines reared at the three temperatures were used for this model. Estimates for composite genetic (d, h, dm) and environmental effects (e1, linear; e2, deviation from linear) and the interactions of genetic and environmental effects (d.e1, d.e2, h.e1, h.e2, dm.e1, and dm.e2) were obtained.

In all models, least-squares procedures were used to estimate model parameters contained in vector **Y** and their variances from the diagonal of their variance covariance matrix S (Mather and Jinks 1982; Lynch and Walsh 1997). The estimates of **Y** and S are obtained as

$$\hat{\mathbf{Y}} = (\mathbf{C}^T\mathbf{V}^{-1}\mathbf{C})^{-1}\mathbf{C}^T\mathbf{V}^{-1}\mathbf{X} \quad (1)$$

and

$$\hat{\mathbf{S}} = (\mathbf{C}^T\mathbf{V}^{-1}\mathbf{C})^{-1}, \quad (2)$$

such that C is the coefficient matrix for the contribution of effects to each line mean, V is the diagonal matrix of the error variances of each line mean, and **X** is the vector of observed line means. All parameters of each model were estimated in a full model to provide a guide to which parameters might be important in reduced models (Mather and Jinks 1982). Goodness of fit of each model was tested by a χ^2, calculated as

$$\chi^2 = \mathbf{X}^T\mathbf{V}^{-1}\mathbf{X} - \mathbf{X}^T\mathbf{V}^{-1}\mathbf{C}\hat{\mathbf{Y}} \quad \text{(Hayman 1958).} \quad (3)$$

(Hayman 1958). The degrees of freedom for this χ^2 is the number of line means minus the number of parameters estimated in the model.

F-statistics were used to evaluate the improvement in the goodness of fit for other parameters (Graybill 1961). This was accomplished by using

$$F_{p_2-p_1,n-p_2} = \frac{(\chi^2_{(n-p_1)} - \chi^2_{(n-p_2)})/(p_2 - p_1)}{\chi^2_{(n-p_2)}/(n - p_2)}, \quad (4)$$

where n is the number of observations, p_1 is the number of parameters in the basic model, and p_2 is the number of parameters of the expanded model.

In the analysis of generation means (or means of line crosses) the loci involved are assumed to be in Hardy-Weinberg and linkage equilibrium. In addition, loci that differ between the two parental lines are assumed to be unlinked. The pa-

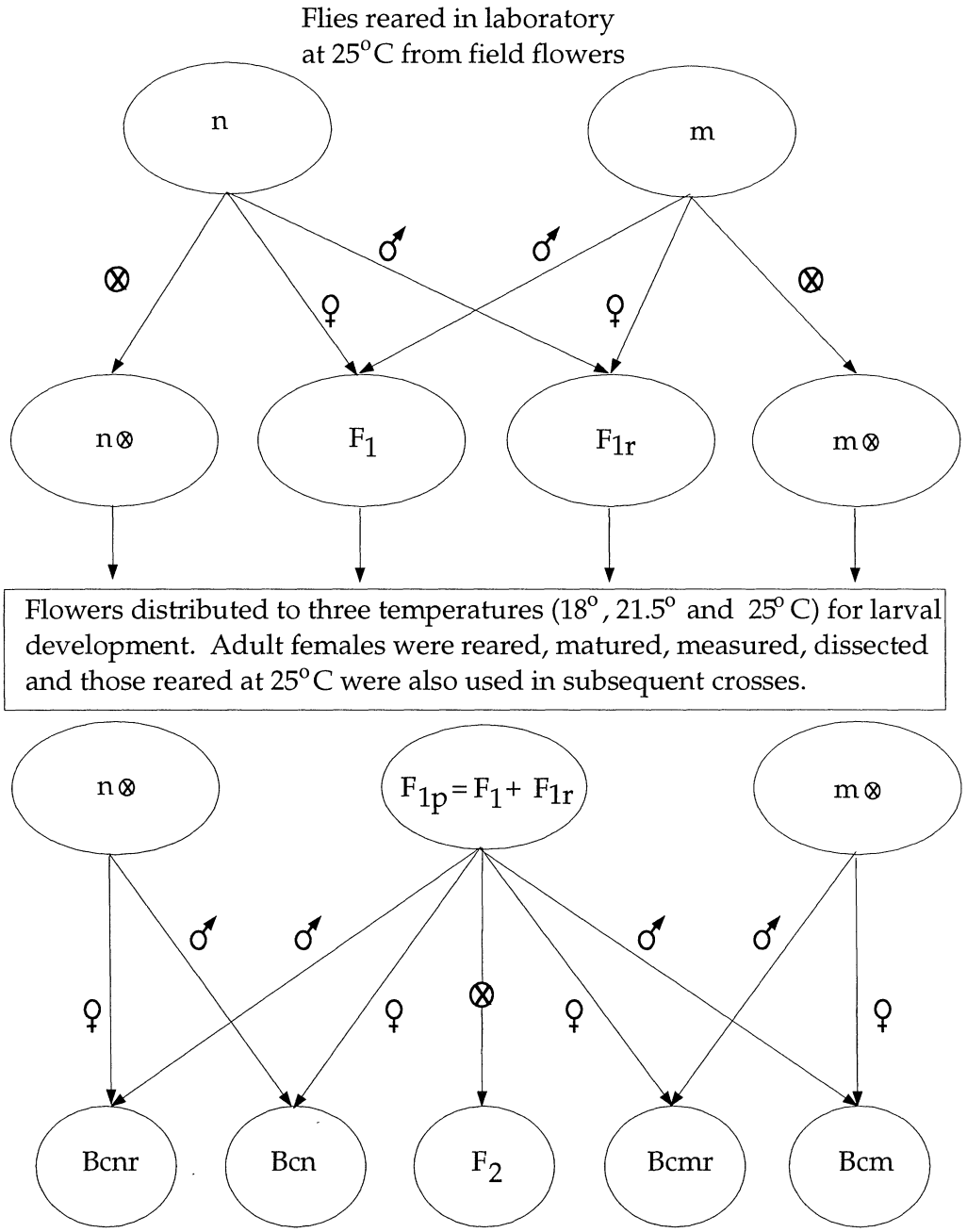

FIG. 1. Crossing scheme for lines used in the genetic and environmental analysis.

rental lines can carry the same alleles but because the analysis estimates the net difference between additive effects, average dominance, and the net directional epistasis, the alleles that are the same only need to differ in frequency (Lynch and Walsh 1997). The composite effects of the model summarize the total effects over all loci such that some alleles will contribute positive and others negative increments to the phenotype. In essence, the models are arbitrary for the number and distribution of loci involved. Estimates of parameters are used to predict the line means as the algebraic sum of the contribution of each parameter associated with the expected genotype of that line (Mather and Jinks 1982).

Field Collections

Two field collections were made one week apart (October 10 and 17, 1996) at one site (BEL; 30°25.155′S, 152°49.425′E; near Bellingen, NSW) for comparison of ovariole number in field-caught females with their progeny reared in the laboratory. We conducted this study because the mean ovariole number of laboratory reared flies of the two populations used in the crosses (**m** and **n**) differed significantly from the numbers estimated from field-caught females in the previous year (Starmer et al., in press). Females from the two BEL collections were returned to the laboratory,

TABLE 2. Statistics for the parental, F_1s, and segregating generations of crosses between **m** and **n**. Larvae were reared at 25.0°, 21.5°, and 18.0°C. ⊗ = self generation of original laboratory reared line. ● = pooled generation. ●● = pooled F_{1p} and F_1●.

25.0°	tl[a]	*n*	(SD)	ova[b]	*n*	(SD)	*r* tl.ova[c]
m	1.030	60	(0.060)	22.91	44	(2.90)	0.336
m⊗	1.005	24	(0.049)	22.30	20	(4.07)	0.695
m●	1.023	84	(0.058)	22.72	64	(3.29)	0.453
n	0.985	49	(0.062)	18.52	46	(3.90)	0.509
n⊗	0.983	22	(0.076)	19.40	20	(4.26)	0.801
n●	0.984	71	(0.066)	18.79	66	(4.00)	0.613
F_1	0.910	46	(0.076)	17.51	43	(3.12)	0.811
F_{1r}	0.885	25	(0.111)	17.25	24	(4.42)	0.897
F_1●	0.901	71	(0.090)	17.42	67	(3.61)	0.849
F_{1p}	1.000	39	(0.063)	22.05	39	(3.84)	0.828
F_1●●	0.936	110	(0.094)	19.12	106	(4.31)	0.874
F_2	0.821	80	(0.089)	14.03	75	(3.53)	0.858
Bcm	0.825	53	(0.095)	14.84	51	(3.58)	0.881
Bcmr	0.800	52	(0.104)	14.18	51	(3.84)	0.892
Bcm●	0.813	105	(0.100)	14.51	102	(3.71)	0.888
Bcn	0.854	50	(0.105)	15.38	50	(4.97)	0.887
Bcnr	0.893	53	(0.098)	16.66	50	(4.21)	0.840
Bcn●	0.874	103	(0.103)	16.02	100	(4.63)	0.867
21.5°	tl	*n*	(SD)	ova	*n*	(SD)	*r* tl.ova
m⊗	0.973	22	(0.093)	22.33	21	(5.01)	0.882
n⊗	1.031	52	(0.055)	23.48	42	(2.96)	0.548
F_1	0.992	44	(0.055)	21.45	40	(3.11)	0.560
F_{1r}	0.934	22	(0.122)	19.87	23	(4.86)	0.856
F_1●	0.973	66	(0.087)	20.87	63	(3.88)	0.756
18.0°	tl	*n*	(SD)	ova	*n*	(SD)	*r* tl.ova
m⊗	0.926	31	(0.094)	19.03	30	(4.74)	0.816
n⊗	0.984	48	(0.089)	20.56	43	(4.60)	0.839
F_1	1.041	36	(0.050)	23.90	31	(3.28)	0.603
F_{1r}	0.983	36	(0.085)	23.10	31	(5.08)	0.843
F_1●	1.012	72	(0.075)	23.50	62	(4.26)	0.741

[a] Mean thorax length in mm.
[b] Mean number of ovarioles.
[c] Correlation between thorax length and number of ovarioles.

measured, dissected, ovarioles counted, and an egg, if present in the uterus, was measured. Recently laid eggs contained in 0–1-day-old field flowers were collected at the same time as the females. These were returned to the laboratory and placed at 18.0°, 21.5°, and 25.0°C on the same day. Adults reared from these flowers were matured in cages with flowers, measured, dissected, and ovarioles counted for comparison with the field-caught females.

RESULTS

The average thorax length of females reared at 25°C varied among lines and each line exhibited a significant ($P < 0.05$) positive correlation between thorax length and ovariole number (Table 2). Among lines (Table 2; m●, n●, F_1, F_{1r}, F_2, Bcm, Bcmr, Bcn, Bcnr), mean thorax length also was correlated positively with mean ovariole number ($\hat{\rho} = 0.964$, df = 7, $P < 0.001$). Therefore, thorax length was used as a covariate in an analysis of covariance to adjust the mean ovariole number for each line. Table 3 lists the least-squares means for parental, F_1s, F_2, and backcross generations reared at 25°C. Mean ovariole number (corrected for thorax length) for parental and self generations of **m** did not differ significantly ($F_{1,61} = 0.00$, $P = 0.98$) so the data for parental and self were pooled. This was also the case with the **n** line ($F_{1,63} = 1.05$, $P = 0.46$). However, the means estimated from the pooled parental data for the two strains **m** and **n** did differ from one another ($F_{1,127} = 25.2$, $P < 0.0001$).

The adjusted means and variances (SE^2) for the nine lines (pool parental in Table 3) were used in a generation means analysis to estimate the composite additive genetic effect (d) in the model ova = m + d, where m is the mean over all populations (model C, Table 4). The regression line resulting from using this estimate is plotted along with the observed generation means in Figure 2. The departure from the regression line and the goodness of fit for the expected generation means ($\chi^2_7 = 8.27$, $P = 0.30$) show the additive model to be adequate but estimates of other parameters (model A, Table 4) indicates the epistatic parameter (j) is significantly

TABLE 3. Least-squares means of ovariole number corrected for correlation with thorax length for the parental, F_1s, and segregating generations of crosses between **m** and **n**. Larvae were reared at 25°C. ⊗ = self generation of original line. ● = pooled generation.

		Unpooled		Pooled parental		Pooled reciprocals	
Line	*n*	ova	(SE)	ova	(SE)	ova	(SE)
m	44	18.345	(0.539)				
m⊗	20	18.180	(0.388)				
m●	64			18.231	(0.330)	18.255	(0.330)
n	46	15.952	(0.535)				
n⊗	20	15.307	(0.361)				
n●	66			15.503	(0.309)	15.520	(0.308)
F_1	43	16.744	(0.356)	16.744	(0.356)		
F_{1r}	24	17.639	(0.476)	17.639	(0.475)		
F_1●	67					17.067	(0.284)
F_2	75	16.563	(0.283)	16.563	(0.283)	16.550	(0.283)
Bcm	51	17.094	(0.336)	17.094	(0.335)		
Bcmr	51	17.320	(0.344)	17.320	(0.344)		
Bcm●	102					17.193	(0.248)
Bcn	50	16.686	(0.333)	16.686	(0.332)		
Bcnr	50	16.601	(0.329)	16.601	(0.329)		
Bcn●	100					16.640	(0.234)

TABLE 4. Estimates of parameters for models (A–F) with and without maternal effects for ovariole numbers at 25°C. Bold numbers indicate significant contributions for that parameter ($\alpha = 0.05$).

	Without maternal effects			With maternal effects		
	A (SE)	B (SE)	C (SE)	D (SE)	E (SE)	F (SE)
m	15.425 (1.335)	16.875 (0.112)	16.875 (0.112)	17.138 (0.257)	16.969 (0.143)	16.994 (0.143)
d	**−1.364** (0.226)	**−1.365** (0.226)	**−1.113** (0.187)	**−0.837** (0.338)	**−0.828** (0.287)	
h	2.912 (3.116)					
i	1.442 (1.315)					
j	**1.606** (0.808)	**1.613** (0.808)				
l	−1.271 (1.899)					
dm				−0.295 (0.244)	−0.291 (0.219)	**−0.769** (0.143)
hm				−0.405 (0.322)	−0.212 (0.231)	−0.239 (0.231)
d.dm				−0.293 (0.366)		
d.hm				0.115 (0.465)		
χ^2	2.53	4.28	8.27	5.08	5.82	14.16
df	3	6	7	3	5	6

different from zero. An *F*-test was used to evaluate the improvement in the goodness of fit for this and other parameters (Graybill 1961). Only the epistatic parameter (j) showed a significant reduction in the χ^2 (model B, Table 4). The *F*-statistic for this test is obtained by comparing the goodness of fit χ^2 of model B ($\chi^2_6 = 4.28$, Table 4) with that of the additive model (model C, $\chi^2_7 = 8.27$, Table 4); $F_{1,6} = (8.27 - 4.28) / (4.28 \div 6) = 5.59$, ($P = 0.056$). An analysis of pooled reciprocals (Table 3) produced essentially the same results with the ova = m + d + j model describing the line means adequately ($\chi^2_3 = 1.84$, $P = 0.64$) and with both d and j contributing significantly ($P < 0.05$).

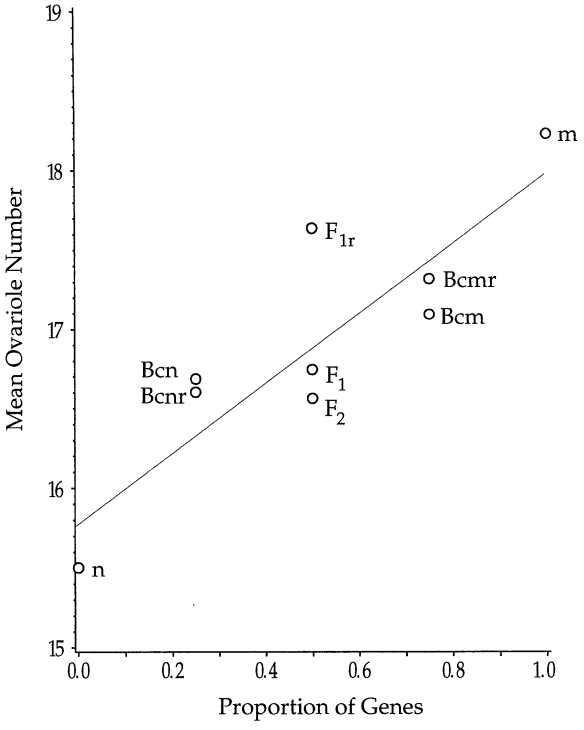

FIG. 2. Observed (points) and expected (line) generation means of ovariole number plotted as a function of the proportion of genes in a simple additive genetic model ova = m + d. The self, F_1, F_2, and backcross generations are defined in Table 1.

Evaluation of the model with maternal effects (Table 4) showed maternal effects and progeny-by-maternal epistasis were not significant. All *F*-tests used to evaluate the significance of adding parameters to model C were not significant ($P < 0.05$). When only maternal additive (dm) and maternal dominance (dh) were used in the model (model F, Table 4), the maternal additive effect was significant but the goodness of fit of the model was not adequate ($\chi^2_6 = 14.2$, $P = 0.03$) to describe the means.

Thorax length and ovariole number were positively correlated ($P < 0.05$) between individuals within lines (r tl.ova, Table 2). The correlation between thorax length and ovariole number was also present between the means of these two variables among lines raised at the three temperatures (25°C: m●, n●, F_1, F_{1r}; 21.5°C: m⊗, n⊗, F_1, F_{1r}; 18°C: m⊗, n⊗, F_1, F_{1r}; $\hat{\rho} = 0.879$, df = 10). The mean ovariole number for

TABLE 5. Least-squares means of ovariole number corrected for correlation with thorax length for the parental, F_1, and F_{1r} generations of crosses between **m** and **n.** Larvae were reared at 25.0°, 21.5° and 18.0°C. ⊗ = self generation of original laboratory reared line. ● = pooled generation.

		Unpooled	Pooled (F_1 and F_{1r})
Line	*n*	ova (SE)	ova (SE)
25.0°			
m●	64	21.236 (0.342)	21.269 (0.344)
n●	66	18.612 (0.331)	18.616 (0.333)
F_1	43	20.073 (0.425)	
F_{1r}	24	21.069 (0.575)	
F_1●	67		20.363 (0.355)
21.5°			
m⊗	21	22.444 (0.587)	22.442 (0.589)
n⊗	42	21.502 (0.424)	21.546 (0.425)
F_1	40	21.040 (0.426)	
F_{1r}	23	21.625 (0.579)	
F_1●	63		21.240 (0.343)
18.0°			
m⊗	30	21.113 (0.499)	21.066 (0.501)
n⊗	43	20.542 (0.410)	20.542 (0.412)
F_1	31	21.188 (0.497)	
F_{1r}	31	22.628 (0.483)	
F_1●	62		21.943 (0.350)

TABLE 6. Estimates of parameters for three models (A–C) for ovariole numbers at different temperatures. Bold numbers indicate significant contributions for that parameter ($\alpha = 0.05$).

	A	B	C
m	20.376 (0.201)	20.423 (0.195)	20.379 (0.196)
d	0.190 (0.320)		
h	**0.864** (0.320)	**0.848** (0.312)	**0.881** (0.312)
dm	**0.609** (0.249)	**0.714** (0.130)	**0.642** (0.132)
e1	**−0.452** (0.201)	**−0.557** (0.154)	**−0.520** (0.154)
e2	**1.597** (0.414)	**1.627** (0.396)	**1.648** (0.396)
d.e1	0.624 (0.320)		**0.551** (0.195)
h.e1	−0.217 (0.320)		
dm.e1	−0.111 (0.249)		
d.e2	−0.011 (0.602)		
h.e2	**−1.504** (0.602)	**−1.439** (0.577)	**−1.471** (0.577)
dm.e2	−0.317 (0.437)		
χ^2	—	10.45	2.46
df	0	6	5

each line was therefore adjusted by using thorax length as a covariate in an analysis of covariance. The resulting least-squares means for line ovariole number at the three temperatures are listed in Table 5. These means and their variances were used to estimate genetic and environmental parameters that might explain the differences in ovariole number (Table 6).

Investigation of several models resulted in an adequate description of the 12 line means by using seven parameters: mean, m; progeny dominance, h; maternal additive, dm; environmental effects (e1, linear; e2, deviations from linear); and interactions of genetic and environmental effects, d.e1 and h.e2). All of these estimates were significant ($P < 0.05$) and the model expectation for each generation mean was close to the observed means ($\chi^2_5 = 2.45$, $P = 0.78$). These results revealed significant dominance but no significant additive effects of the progeny genotypes. However, maternal genotypes had an additive effect. In addition, environmental effects were strong, including a nonlinear environmental effect (e2) that interacted with progeny dominance (h.e2). The e2 effect is apparent in Table 5 as a higher ovariole number at the intermediate temperature (21.5°C).

Analysis of asymmetry in ovarioles (ovasym ± SE) showed the parental strains **m** (1.094 ± 0.101) and **n** (0.909 ± 0.099) to be statistically equivalent ($F_{1,128} = 1.71$, $P = 0.19$). Egg volume (mm^3 ± SE) for strain **m** (0.0133 ± 0.0004) was statistically equivalent ($F_{1,51} = 0.56$, $P = 0.46$) to strain **n** (0.0129 ± 0.0004). Therefore, the genetic analysis of these two traits was not carried out.

The comparison of field-caught females with laboratory reared progeny of the field-caught female generation is listed in Table 7. Two features of these data are apparent. The field-collected females have significantly fewer ovarioles (means adjusted for thorax length) than the laboratory-reared progeny regardless of larval rearing temperature (1st week $F_{1,58} = 31.3$, $P < 0.0001$; 2nd week $F_{1,94} = 17.8$, $P < 0.001$) and the two collections of field females (one week apart) have significantly different ovariole numbers ($F_{1,56} = 9.4$, $P = 0.003$) but the flies reared in the laboratory are not different between weeks ($F_{1,96} = 0.13$, $P = 0.72$).

DISCUSSION

The relationship between ovariole number and fitness has not been well established within any *Drosophila* species. Wayne et al. (1997) studied 45 isogenic lines of *D. melanogaster* derived from nature but found no correlation between ovariole number and fitness. This result differed from that found by David (1970) and Boulétreau-Merle et al. (1982), who observed a positive correlation between ovariole number and the maximum daily rate of oviposition. The difference between these studies could be based on differences in how female fecundity was measured and further experiments are required to explain the discrepancy (Wayne et al. 1997).

The wide range of ovariole number (one per ovary to as many as 100 per female) in the Hawaiian drosophilids fits a pattern of low fecundity to high fecundity as the ovariole number increases. This pattern is coincident with the variation in the abundance and nutrient quality of the larval niche in which rich and abundant substrates are hosts to species with a large number of ovarioles and nutritionally poorer substrates are hosts to species with a lower number of ovar-

TABLE 7. Statistics for females collected at Bellingen one week apart. Females reared from flowers incubated at three temperatures (25.0°, 21.5°, and 18.0°C) and collected at the same time as the field-collected flies are listed beneath the first and second collection (b1 = October 10, 1996, and b2 = October 17, 1996).

	tl[a]	*n*	(SD)	ova[b]	*n*	(SD)	*r* tl.ova[c]	adj ova[d]	(SE)
b1									
field	0.952	19	(0.060)	15.84	19	(2.34)	0.493	15.390	(0.510)
25.0°	0.879	17	(0.076)	16.33	15	(3.48)	0.894	18.939	(0.599)
21.5°	0.837	19	(0.077)	15.22	18	(3.00)	0.925	19.310	(0.589)
18.0°	0.868	20	(0.057)	17.44	9	(3.88)	0.747	19.828	(0.757)
b2									
field	0.945	47	(0.081)	18.05	40	(3.99)	0.793	17.454	(0.353)
25.0°	0.970	18	(0.040)	20.75	16	(2.46)	0.737	19.562	(0.560)
21.5°	0.997	21	(0.064)	21.29	21	(4.04)	0.863	19.080	(0.506)
18.0°	0.984	21	(0.078)	22.15	20	(3.77)	0.699	20.332	(0.511)

[a] Mean thorax length in mm.
[b] Mean number of ovarioles.
[c] Correlation between thorax length and number of ovarioles.
[d] Mean number of ovarioles adjusted for thorax length.

ioles (Kambysellis and Heed 1971). Flower-breeding species are on the lower end of the "fecundity potential" spectrum where *Scaptomyza* (*Exalloscaptomyza*) *caliginosa* Hardy (breeds in morning glory blossoms on the island of Hawaii) has two ovarioles per female, and laboratory studies show lifetime reproductive output of 8 to 12 progeny per female (Starmer and Wolf, unpubl. data). *Drosophila hibisci* breeds in larger flowers and has a higher number of ovarioles, ranging from means of 10 to 20 in natural populations over a latitudinal range of 13.6° (Starmer et al., in press). The generation means analysis reported here was directed toward understanding the potential genetic and environment determinants of ovariole number between populations of *D. hibisci*. A within population analysis would be valuable for comparison but has yet to be conducted.

The inheritance of ovariole number in *D. hibisci* at 25°C is adequately described by an additive model but additive-by-dominance epistasis significantly improves the fit of the model. This result indicates that digenic epistasis probably is involved in determining the ovariole number of *D. hibisci*, at least in this interpopulational study. The relationship of the additive (d) and epistatic (j) components is such that they are of opposite sign but equal in magnitude. The consequence of this form of epistasis is to reduce the variance in the F_2 and backcross generations and to reduce the genetic variation on which natural selection can operate. For example, in a hypothetical case, if only two loci each with two alleles are responsible for the phenotype (the actual number of loci is not known in our study), this would produce a 14:1:1 phenotypic ratio in the F_2 of a dihybrid cross and a 3:1 ratio in the backcross generations. This is analogous to classical dominant epistasis that produces a 12:3:1 ratio of phenotypes in the F_2 of a dihybrid cross. Linkage among additive genes also might account for deviations from linearity. An adequate test for linkage would require additional segregating generations (e.g., F_3, F_4).

Studies of other species of *Drosophila* have also uncovered significant epistatic influences on ovariole number. R'Kha et al. (1991) conducted a similar genetic analysis by crossing two populations representing two closely related species in the D. *melanogaster* species complex (*D. sechellia* and *D. simulans*). They raised the parental, F_1, and backcross generations on medium with and without the addition of morinda fruit. Both conditions revealed strong genetic components in the determination of ovariole number. We reanalyzed their data using generation means analysis and confirmed their findings of significant additive effects and no dominance. In addition, we detected a significant additive-by-additive epistatic component when the means for populations reared without the addition of morinda fruit were used in the analysis. Chakir et al. (1995) also found evidence of epistatic effects on ovariole number in *D. melanogaster* strains collected in France and the Congo. Their study analyzed the effects of chromosomal substitutions between the two populations and found significant interactions between chromosomes 2 and 3. Chakir et al. (1995) compared their results with other investigations (Thomas-Orillard 1975, 1982) on French and Japanese populations and found them consistent in terms of chromosomal involvement. They suggest that genetic determination of ovariole number is due to the action of homologous genes in these cases and that the homology may extend to other species. Our results are similar only with respect to the mode of gene activity because we have no data on chromosomal effects.

In *D. hibisci*, parental and F_1 generations raised at different temperatures revealed dominance (h), maternal effects (dm), strong linear and nonlinear environmental components (e1, e2), and genotype-by-environment interactions (d.e1 and h.e2). A significant maternal effect on ovariole number also has been reported in *D. melanogaster* by Thomas-Orillard (1984). She found that a picornavirus in the cytoplasm is responsible for an increase in ovariole number as well as affecting other quantitative traits such as developmental time and fresh weights of females. Delpuech et al. (1995) also reported nonlinear effects of temperature on ovariole number in temperate and tropical populations of *D. melanogaster*. In both studies, ovariole number was higher at intermediate temperatures than at higher and lower temperatures.

Because the segregating generations were not raised at the different temperatures, it was not possible to estimate digenic epistasis or interactions of epistatic effects with environmental effects. It is nevertheless significant that nonlinear terms involving genetic effects and environmental effects are prevalent in the analysis of parents and their F_1s. These results in conjunction with our observations on differences between ovariole number of laboratory-reared females and field-caught females (corrected for body size) indicate the phenotype is very sensitive to environmental change. Even females collected from the same site one week apart showed significant changes in ovariole number (Table 7). This was also true for sites at which *D. hibisci* were collected almost one year apart (Starmer and Barker, unpubl. data). Boulétreau (1978) found results similar to ours for the ovariole number of *D. melanogaster* females dissected from field collections and for their progeny reared in the laboratory. She concluded that field-collected females have fewer ovarioles because of a trade-off with selective pressures for survival.

Fly reproductive potential undoubtedly is modified by environmental fluctuations. The analysis of data from *D. hibisci* shows that temperature of larval development is an important influence on ovariole number (Table 6). However, other factors that were not tested that could be important include: (1) larval density or competition; (2) temperature fluctuations experienced during larval development; (3) flower nutrient quality; (4) flower moisture content; and (5) influence of nematode (*Howardula* sp.) parasites that are found in the natural populations. For example, Kambysellis et al. (1979) showed the ovariole number of *D. mimica* varied over seasons and attributed this variation to changes in body size that most likely resulted from larval competition.

We agree with Hard et al. (1992, 1993) that "models assuming strictly additive genetic effects, even if appropriate at the within-population level, may tell us little about the actual genetic divergence of populations." We might add that assuming other nonlinear effects such as genotype-by-environment interaction can be ignored may also be misleading. The nature of the genetic architecture and its relationship to the environment is the basis for understanding and evaluating theories on evolution. If Sewall Wright's shifting balance theory is plausible, then complex characters will be con-

trolled by epistatic genes and influenced by other nonlinear components of the genetic system. Revealing the nature of the genetic structure is thus an important step in evaluating the process leading to adaptation and divergence.

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Corresponding Editor: L. Stevens