Artificial selection for a secondary sexual character in males of *Drosophila silvestris* from Hawaii

(morphometrics/evolution/population genetics/speciation)

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ABSTRACT *Drosophila silvestris* is endemic to the rain forests of Hawaii Island. In populations from the northeast side, the male foreleg tibia bears about 100 long cilia; these are used as a brush to stimulate the female during courtship. Cyclical family selection for high and low cilia number was carried out on progeny of lines started from single wild isofemales collected at a single site. Significant response in both directions was obtained; in the third generation the lines differed by a mean of 25 cilia. Despite much effort to save them, both lines died out without further advance; the low cilia number line died after five and the high cilia number line died after nine generations. The data are compatible with the view that the cilia character is polygenic and is maintained in nature by a stabilizing sexual selection that also favors a polygenic heterozygous state. Although selection in these experiments resulted in dysgenesis, such a result is not inevitable. Disorganization and reorganization of such a genetic system following a population bottleneck might be important as a predisposing condition for the emergence of a specifically novel and possibly isolating mode of courtship.

Highly speciose lineages of insects and many other animals often display elaborate secondary sexual characters in males (1–3). Alteration of the system of sexual selection may be among the first crucial types of genetic change in the process of species formation in such forms (4, 5). This may be especially pronounced in cases wherein founder effects are implicated in the origin of species (6).

Although many aspects of microevolutionary change by mutation, recombination, and selection have been elucidated by population genetics, experimental access to the crucial dynamic stages of speciation has been difficult. Selected species groups of Hawaiian *Drosophila* serve as valuable observational and experimental material for such studies (7, 8). The present paper continues the scrutiny of a particular character, the tibial cilia number of the male *Drosophila silvestris*. Cilia are elongated bristles that are used by males to brush the female's abdomen during courtship and appear to be under epigamic sexual selection. They are lacking in females. Confined to the Island of Hawaii, *D. silvestris* is evidently a newly formed species. Between the two sides of the island, the cilia character differs geographically (8) and genetically (9). Further, it displays a novel pattern of genetic instability in a population on the new lava flows of the currently active volcano, Kilauea (ref. 10 and unpublished data). The present paper reports the results of artificial selection experiments that have been used to explore further biological aspects of the pattern of phenotypic and genotypic variation in this same population.

MATERIALS AND METHODS

Experimental Material. We report here experiments on *D. silvestris* lines established by isolation and laboratory culture of the descendents of single females captured in nature in 1979 at Olaa Tract, Hawaii Volcanoes National Park (for details of the collecting site, see ref. 10). Simultaneously with the capture of the wild females, a base sample of 21 wild males was also obtained. Using a method described earlier (8), the tibia of the right leg of each male was removed and mounted. Under a compound microscope, counts were made of the number of cilia present. On the dorsal surface of the tibia, these enlarged hairs are deployed in three rows, developmentally known as row 5, row 5a, and row 6 (11). The number in each row was recorded separately.

Selection Procedure. Artificial selection for high and low cilia number was begun by selecting the high and low families from among the F_1s produced by each of 5 different wild females collected in 1979. Ten or 20 males were used to estimate the mean number for each family. Following selection of one high and one low family, a randomly mated mass F_2 was reared and 10–20 individual pairs of virgin flies were made up. These provided a series of F_3 families for the second cycle of selection. Cilia were counted from about 10 males from each family and these were combined to give the figures for each generation as recorded in Table 2. This process was continued through F_9 for high selection and F_5 for low selection. Despite relaxed selection in alternate generations, reproductive fitness of the experimental lines was seriously impaired. Many pairs were infertile, and after F_5 (March 1981) the low line could no longer be maintained since the adult flies, although appearing vigorous, did not reproduce. The same phenomenon occurred in the high line at F_9, after which demise of this line occurred. Of 25 low pairs made up in F_5, only 5 gave progeny; of 32 high pairs in F_9, only 5 gave progeny. The selected pairs gave only a very small mass culture and the lines were lost. Isofemale lines of this species reared by routine mass methods of laboratory culture without being subjected to special selective procedures normally survive well and tend to retain a consistent mean cilia number. The data for a laboratory stock collected as an isofemale in January 1977 are given in Table 1. The data appear homogenous. The largest difference (tibial row

Table 1. Stability of tibial cilia number in a laboratory stock of *D. silvestris* (U28T2, Kilauea Forest Reserve, Hawaii)

Sample		Tibial cilia		
Date	*n*	Row 5	Row 5a	Row 6
Oct. 1978	20	28.20 ± 0.69	24.15 ± 1.20	29.95 ± 0.51
Feb. 1980	20	30.10 ± 0.78	23.20 ± 1.91	30.60 ± 0.54
Feb. 1981	10	29.70 ± 1.00	23.60 ± 1.35	30.80 ± 0.36
Oct. 1983	50	29.08 ± 0.38	21.38 ± 0.90	29.90 ± 0.35

Values are given as mean ± SEM.

Table 2. Selection for high and low tibial cilia number in males of D. silvestris from Olaa tract, Hawaii

| Sample | n | Tibial cilia | | | |
		Row 5	Row 5a	Row 6	Total
P_1 wild	21	35.24 ± 0.77	35.38 ± 1.85	30.48 ± 0.53	101.10 ± 2.59
F_1 high	20	34.00 ± 0.64	34.30 ± 1.42	30.80 ± 0.55	99.10 ± 1.90
F_3 high	40	36.33 ± 0.51	43.20 ± 1.07	30.90 ± 0.41	110.43 ± 1.41
F_5 high	39	37.72 ± 0.53	47.05 ± 0.84	30.38 ± 0.32	115.15 ± 1.14
F_7 high	30	35.77 ± 0.62	46.47 ± 0.96	29.83 ± 0.34	112.07 ± 1.39
F_9 high	43	36.63 ± 0.47	43.30 ± 0.78	29.86 ± 0.26	109.65 ± 1.02
F_1 low	10	28.20 ± 0.73	25.00 ± 1.70	30.10 ± 0.59	83.30 ± 2.32
F_3 low	35	29.51 ± 0.50	24.91 ± 0.80	30.60 ± 0.38	85.03 ± 1.33
F_5 low	43	31.16 ± 0.46	29.42 ± 1.06	32.58 ± 0.38	92.93 ± 1.47

Values are given as mean ± SEM.

5a for October 1978 vs. October 1983) gives a Student's $t = 1.71$; degrees of freedom = 68; $P = 0.10$.

RESULTS

The data for each of the three rows of cilia and the totals are given for both high and low lines in Table 2. Student's t tests for the comparisons of crucial samples are given in Table 3, along with estimations of the level of statistical significance for the difference between each pair of compared samples. Perusal of the data show that by F_3 the high line had made a very significant advance of 9.3 in total cilia, a level that was essentially maintained thereafter. Of particular interest is the fact that all of the total response was concentrated in row 5a; rows 5 and 6 were insensitive to selection for high cilia number (Table 3). Accordingly, the summary diagram (Fig. 1) shows the changes in row 5a only.

In the low selection line, advance is likewise immediate and large but decays by almost one-half in later generations. Unlike selection for high number, selection in a low direction evokes a response from all three rows of cilia although the major response is by row 5a, as was also the case in the high selection lines.

DISCUSSION

Directional selection for cilia number in males results in a difference of about 25 cilia between high and low lines. This shift occurs quickly, within one or two generations. After this, there is little or no advance; on the contrary, despite continued artificial directional selection, there is a tendency of both high and low lines to move back towards the mean of the original wild population.

Most of the selective advance involves row 5a, the so-called "middle row" of tibial cilia (8, 11). This row of cilia is absent in silvestris males from the southwest side of the island (Kona side) as well as in all other members of a group of five species (the planitibia subgroup) from Hawaii and the adjacent islands of Molokai, Maui, and Oahu (7, 8). Thus, the 30-odd cilia of row 5a are a phylogenetically new embellishment of the ciliary brush in a region of the leg that is elsewhere virtually bare.

Crosses with flies lacking cilia in row 5a reveal (in preliminary analyses) a genetic basis of three segregating units, one X-linked and two autosomal (9). In the present experiments, selection causes a change in the mean of these cilia in particular; this suggests that the genetic basis of this new evolutionary change is still polymorphic in natural populations on the northeast (Hilo) side of the island. This same conclusion was drawn from observations of temporal genetic shifts in this character at Olaa Tract over 4 years (10) and is compatible with the variance observed in F_1s of crosses with flies from the southwest (Kona) side of the island (9).

Selection in either direction away from the population mean produces dysgenic lines that cannot be maintained in the laboratory. This suggests that, in the natural population of silvestris, some form of stabilizing selection is operating to maintain an optimal number of cilia. What are the components of this lowered fitness? We suggest that sexual selection based on female choice (12) may be the stabilizing agent. Thus, females may favor males with cilia numbers near the mean of the population. As was originally suggested by Bösiger (13), the sexual selection system may operate by selecting for high-fitness heterozygotes, thus providing for the maintenance of genetic variability for this character. The data on the nature of the lowered fitness in the selected lines, however, are not robust enough to eliminate the possibility that the opposing force is simply correlated natural selection for viability, due to an inbreeding depression accompanying selection.

Most of the large picture-winged species of Drosophila from Hawaii display elaborate secondary sexual characters in males. If variability can be maintained in natural popula-

Table 3. Significance of responses to selection of high and low tibial cilia number of males of D. silvestris

| Sample comparison | Tibial cilia | | | | | | | | | | | | |
| | Row 5 | | | Row 5a | | | Row 6 | | | Total | | | |
	Difference ± mean cilia	t	P	Difference ± mean cilia	t	P	Difference ± mean cilia	t	P	Difference ± mean cilia	t	P	df
1979 wild vs. F_3 high	+1.1	1.24	0.9	+7.8	3.94	0.001	+0.4	0.39	0.7	+9.3	3.42	0.001	59
1979 wild vs. F_9 high	+1.4	1.32	0.2	+7.9	4.32	0.001	−0.6	1.13	0.3	+8.6	3.36	0.01	54
F_3 high vs. F_5 high	+1.4	1.90	0.1	+3.9	2.82	0.01	−0.5	1.01	0.3	+4.7	2.04	0.05	77
F_3 high vs. F_9 high	+0.3	0.14	0.9	+0.1	0.14	0.9	−1.0	0.90	0.3	−0.8	0.54	0.6	73
1979 wild vs. F_1 low	−7.0	5.73	0.001	−10.4	3.53	0.001	−0.4	0.43	0.7	−17.8	4.34	0.001	29
1979 wild vs. F_5 low	−4.1	4.78	0.001	−6.0	2.90	0.01	+2.1	3.18	0.01	−8.1	2.95	0.01	62
F_1 low vs. F_5 low	+2.9	2.89	0.01	+4.4	1.88	0.1	+2.4	2.85	0.01	+9.6	2.96	0.01	51

t = Student's t statistic; df = degrees of freedom.

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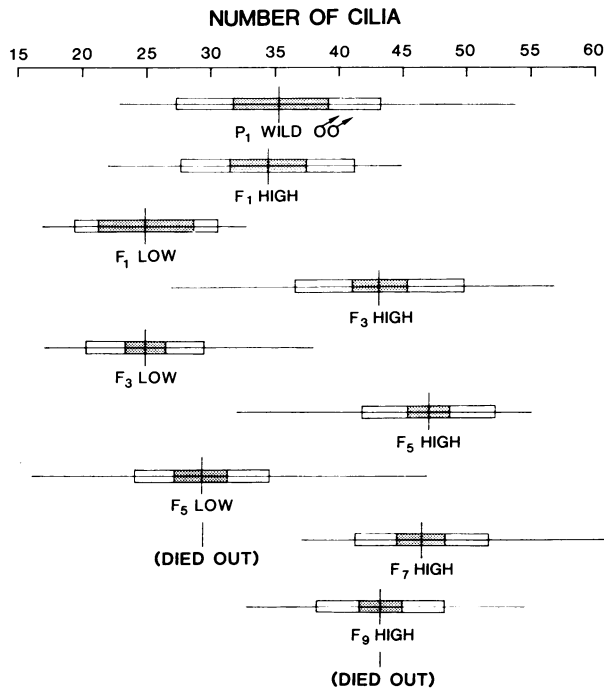*Proc. Natl. Acad. Sci. USA 81 (1984)*   3917

FIG. 1.   Effect of selection for high and low tibial cilia number in male *D. silvestris*. The diagrams give cilia numbers in row 5a and represent mean, range, standard deviation, and 95% confidence limit (stippled). For details see Table 2 and text.

tions in the manner suggested above, much of the exuberance of speciation in Hawaiian *Drosophila* could be explained by shifts in the genetic basis of secondary sexual characters of males. Such shifts would be especially expected in a species that serially colonizes new lava flows by suc-

cessive founder effects. Thus, sexual selection continually demands the maintenance of high-fitness sex partners, and the genetic basis of this fitness may be destabilized by genetic recombination following the founder event. This is essentially an intrapopulational process occurring as a population becomes allopatrically separated. The very same process may ultimately result in interpopulation reproductive isolation, a side effect of sexual selection that nevertheless may contribute to the process of speciation.

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