Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6904–6907, November 1984
Population Biology

Inheritance of a secondary sexual character in *Drosophila silvestris*

(quantitative genetics/sexual dimorphism/evolution/speciation/Hawaiian *Drosophila*)

HAMPTON L. CARSON* AND RUSSELL LANDE†

*Department of Genetics, University of Hawaii, Honolulu, HI 96822; and †Department of Biology, University of Chicago, Chicago, IL 60637

Contributed by Hampton L. Carson, July 16, 1984

ABSTRACT **Reciprocal crosses were carried out between laboratory stock specimens obtained from two races of *Drosophila silvestris* from the island of Hawaii that differ in a quantitative secondary sexual character. The race from the Hilo side of the island has a novel attribute, consisting of an extra row of cilia on the tibia of males, which is used during courtship. With regard to this character, sex-linked genes contribute about 30% of the difference, and the remaining 70% of the difference between the races is produced by genes on at least two autosomes. The novel character appears to have been the outcome of altered sexual selection in the Hilo-side race. In an altered genetic environment, resulting from a founder event or random genetic drift, sexual selection may take a new direction. Such a shift may serve as a model for incipient speciation.**

Male secondary sexual characters are among the most rapidly evolving morphological traits of higher animals, and closely related species are often distinguished taxonomically by differences in male morphology (1). In such taxa, morphological divergence and reproductive isolating barriers between species may often originate by sexual selection via female mating preferences (2–6). This appears to apply to many of the several hundred species of large Hawaiian *Drosophila*, and sexual selection may have been an important factor in their proliferation (7, 8). Here we examine the genetic basis for the origin of a novel secondary sexual character by analyzing crosses between two races of *Drosophila silvestris*.

This species is endemic to the volcanic island of Hawaii, which is geologically very young; it has no lava flows older than 0.5 million years (9). Males from populations on the south and west side of the island ("Kona side") have two rows of long cilia on the margins of the tibia of the foreleg. These are lacking in females. Between the rows is a dorsal bare area having at most two cilia. In populations to the north and east ("Hilo side"), this bare area is occupied by an additional row of about 20–30 cilia (10). Reciprocal crosses between the Kona and Hilo races give fully viable and fertile progeny of both sexes (11).

The extra cilia are not a trivial character; during a crucial stage in courtship, they are used to brush vigorously on the dorsal surface of the female's abdomen (12). Related species in the same subgroup resemble the Kona-side (bare) race of *D. silvestris*, having only two rows of cilia. Accordingly, the extra cilia on Hilo-side (hairy) males represent a novel and very recent evolutionary embellishment of a secondary sexual character. This provides the special focus for the following analysis of its genetic basis.

METHODS AND RESULTS

There are two basic methods for analyzing the genetic basis of phenotypic differences between closely related taxa that can be crossed to produce fertile hybrids. First is the classical genetic technique using mutations with known positions in the genome of one taxon to map the positions of genes producing differences between taxa in the trait(s) of interest. This method has been applied recently by Coyne (13) to analyze three sibling species in the *Drosophila melanogaster* group that differ morphologically only in the shape of the male genitalia. He showed that the species differences are polygenic, with contributions from at least one gene on every chromosome or on each arm of the major chromosomes.

The second method is purely biometrical and was developed originally by Wright (chapter 15 in ref. 14) to estimate the effective (or minimum) number of freely segregating genetic factors contributing to an extreme difference in a quantitative character between two inbred (homozygous) lines by assessing the amount of genetic variance segregating in F_2 or backcross populations. It has since been extended to the analysis of crosses between wild (or genetically variable) taxa (15). The biometrical method has been used to show that the major difference in head shape between the closely related Hawaiian *Drosophila* species *D. heteroneura* and *D. silvestris* is polygenic, with a minimum of about six to eight genes involved (15–17). In the absence of available mutant stocks or known genetic markers to distinguish all the chromosomes in the two races, we have employed the biometrical method to investigate the inheritance of the morphological difference between the races of *D. silvestris* described above.

Reciprocal crosses between two stocks collected from Kahuku Ranch, South Kona District (isofemale line U26B9), and Kilauea Forest Reserve, Puna District (isofemale line U28T2), representing respectively the bare and hairy races of *D. silvestris*, were performed between 1978 and 1983 (see Fig. 1). Methods of rearing specimens, mounting legs and counting cilia are given in ref. 10. All counts treated here refer to the middle (dorsal) row of cilia only. During the period of the experiment, the mean values of cilia counts within the stocks changed very little. Substantial genetic variance within the hairy stock (see below) creates real differences between families, which invalidates the application to family data of conventional statistical tests for homogeneity of means and variances. More conservative tests using within-family means and variances as data points failed to disclose significant differences between sets of families produced by the same crossing scheme at different times. Therefore, data collected at different times was pooled whenever appropriate for purposes of the analysis.

Cytoplasmic Effects. There is a conspicuous difference in the distributions of reciprocal F_1 populations (Fig. 1), which in principle could be produced either by maternal (or cytoplasmic) effects or by sex-linked genes, or both. To determine the amount of difference in reciprocal F_1 populations caused by maternal or cytoplasmic effect, comparisons were made between the average values of families with different cytoplasms but the same expected composition of nuclear genotypes. There are three such tests: two of them are comparisons between averages of male progeny from F_1 females backcrossed to the same parental stock; and, on the assumption that the Y chromosome is genetically inert with respect

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

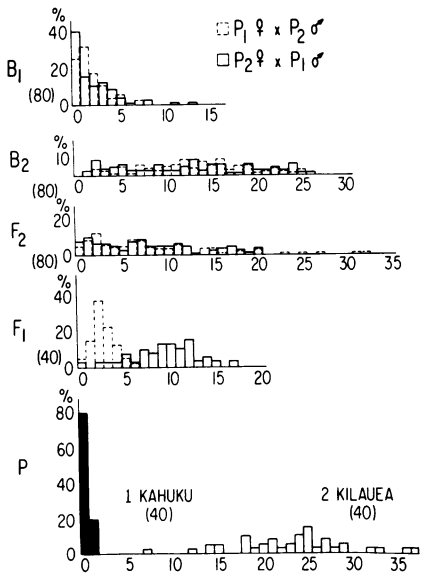

FIG. 1. Distributions of cilia counts for male progeny in crosses between stocks of the bare and hairy races of *D. silvestris*. Sample sizes are given in parentheses. Families containing 20 individuals were pooled to produce the distributions, except for the parental stocks from which individuals were sampled at random. Backcross distributions ($B_1 = F_1 \times P_1$ and $B_2 = F_1 \times P_2$) are each composed of two families from F_1 males and two families from F_1 females, with parents from the appropriate reciprocal cross indicated by dashed or solid lines. F_2 families were produced by sib mating within F_1 families.

to this character, a comparison of the averages of reciprocal F_2 males also can be used. Data for these tests are presented in Table 1. Sampling variances for the average values of the populations were computed from the variance between families divided by the number of families, except for the F_2 populations for which the averages of pairs of related families were taken as data points. In neither of the backcross comparisons, nor in the comparison of reciprocal F_2 populations, was a significant cytoplasmic effect observed. In conjunction with the lack of cytoplasmic effect in the backcross tests, the similarity of reciprocal F_2 populations, which also differ in their Y chromosomes, indicates that the Y chromosome has no influence on this character. Pooling the three tests gives an average effect of hairy versus bare cytoplasm of 0.50 ± 1.78 cilia, which is not significantly different from zero. Therefore, we can conclude that the difference between reciprocal F_1 crosses depicted in Fig. 1 is caused by genes linked to the X chromosome.

Sex-Linked Genes. The magnitude of the effect of X-linked genes can be estimated by the difference between F_1 males from reciprocal crosses, assuming no maternal or cytoplasmic effect and no Y-chromosome effect. The averages of F_1 families from each of the reciprocal crosses and the average values within the parental stocks are shown in Table 2. The difference in the effect of X chromosomes from the hairy and bare races is 6.68 ± 0.71 cilia, which can be contrasted with a total difference of 22.70 ± 1.00 cilia between the pure parental stocks. Thus X-linked genes account for 29.4% ± 3.4% of the difference between the races.

Transformation of Scale. For biometrical analysis of the effective number of autosomal factors contributing to the difference between the Kahuku and Kilauea stocks, it is desirable to choose a scale of measurement on which all of the genetic variance is additive. Because some of the distributions in Fig. 1 are truncated by the lower limit for expression of the trait at zero cilia, a suitable criterion for additivity of autosomal genes in the crosses is that the average B_2 progeny fathered by F_1 males (from either reciprocal cross) should be intermediate between the average of the P_2 stock and the average F_1 progeny obtained from P_2 females × P_1 males. Males from these three populations, with distributions that are not truncated by the threshold at zero cilia, all have X chromosomes from the hairy stock and differ only in the genetic composition of their autosomes.

Wright (chapter 10 in ref. 14) described a method of determining a scale transformation for a set of populations that tends to equalize the variances of populations with different mean values; in many cases this transformation also produces approximate additivity of mean values in crosses between populations. Wright's method consists of linear regression of the standard deviations of the populations on their mean values. The intercept of the regression line on the axis of mean values becomes the constant L in the scale transformation $x' = \log(x - L)$. Because the variance increases with the mean in F_1 and P_2 populations (Fig. 1), which are not segregating for genetic differences between the bare and hairy stocks, we applied Wright's method to five P_2 families (of 10 individuals each) and six F_1 families (four containing 10 individuals and two containing 20 individuals) from P_2 females × P_1 males. The least-squares regression line obtained by weighting each point by the degrees of freedom in the family was $s = 0.1844\bar{x} + 1.8383$, which indicates the scale transformation $x' = \log(x + 9.9691)$. We used natural logarithms (base e) to obtain (Table 3) the grand means and average variances within families on the transformed scale for the P_2 stock and for the appropriate F_1 and B_2 populations.

The degree of additivity in the crosses can be assessed by the statistic

$$D = \bar{\bar{x}}'_{B_2} - \frac{1}{2}(\bar{\bar{x}}'_{F_1} + \bar{\bar{x}}'_{P_2}),$$

which is expected to be zero with perfect additivity. This statistic has a sampling variance equal to the weighted sum of the sampling variances of the three population means (computed as above), with weights being the squares of coefficients in the preceding formula. From the data summarized in Table 3, $D = -0.019 \pm 0.045$, which is not significantly different from zero. On the transformed scale of measurement there is a close approach to perfect additivity of auto-

Table 1. Tests for maternal or cytoplasmic effects on cilia counts in male progeny

	Cilia, no.	
	P_1 male × P_2 female	P_2 male × P_1 female
B_1	2.00 ± 0.10	2.85 ± 1.10
B_2	11.53 ± 0.28	12.43 ± 3.43
F_2	8.13 ± 3.88	7.86 ± 0.64

Reciprocal backcrosses are from F_1 females, with grand means and SEMs based on two families of 20 individuals. Values for reciprocal F_2 populations are each from two pairs of related families of 20 individuals with the same grandfather.

Table 2. Effect of X-linked genes on cilia counts in male progeny

	Cilia, no.	
	P_1 male × P_2 female	P_2 male × P_1 female
F_1	2.48 ± 0.13	9.15 ± 0.70
	P_1	P_2
Parental stocks	0.20 ± 0.16	22.90 ± 1.00

For reciprocal F_1 populations, grand means and SEMs are based on two families of 20 individuals. Means and SEMs for the parental stocks P_1 and P_2 are based on 40 randomly sampled individuals.

Table 3. Grand means and variances for cilia counts of male progeny

	$\bar{\bar{x}}'$	$\bar{s}^2_w{}'$	n	N
P_2	3.4243	0.03409	5	50
F_1	2.8858	0.04023	6	80
B_2	3.1362	0.05290	19	230

Grand mean, $\bar{\bar{x}}'$, and average variance within full-sib families, $\bar{s}^2_w{}'$; for cilia counts of male progeny on the transformed scale $x' = \ln(x + 9.9691)$ in the P_2 stock, in F_1 families from P_2 females × P_1 males, and in B_2 families fathered by F_1 males (from either reciprocal cross). Number of families and total individuals in each population are denoted respectively by n and N. Calculations employed weighting by family size for $\bar{\bar{x}}'$ and by degrees of freedom for $\bar{s}^2_w{}'$.

somal genes in this cross because the absolute magnitude of D is only 3.5% of the range between the F_1 and P_2 populations.

Heritable Variation in the Hairy Race. Analysis of variance for the five families from the Kilauea stock, totalling 50 individuals reared in the same generation, allows an estimate to be made of the heritability of the character, h^2, which is the proportion of the total phenotypic variance produced by additive effects of genes. Utilizing formulae given by Falconer (chapter 10 in ref. 18), we estimate the heritability on the transformed scale of measurement to be $h^2 = 0.32 \pm 0.31$. A similar analysis for another population of the hairy race from Olaa Tract, Hawaii Volcanoes National Park, using six families produced from wild-caught females totalling 116 individuals (and assuming single male paternity of each family), yields an estimate of $h^2 = 0.74 \pm 0.33$. Together these estimates give an average heritability within populations of the hairy race of $h^2 = 0.53 \pm 0.23$. These estimates are based on the assumptions of no maternal effects, random mating, and completely additive genetic variation within populations on the transformed scale of measurement. Although the estimates are not precise, they do indicate that the phenotypic variation within populations of the hairy race is moderately to highly heritable.

Autosomal Genes. Application of the biometrical method to estimate the minimum number of freely segregating autosomal genes contributing to the difference between the races must allow for considerable heritable variation within the parental populations. On the assumption that, on a transformed scale of measurement, all genetic variance within and between populations is additive, and in the absence of maternal effects, the contribution of autosomal genes can be separated from that of X-linked genes by comparing means and variances of male progeny in the parental stocks, reciprocal F_1s, and backcrosses fathered by F_1 males (from either reciprocal cross). Letting the contributions to the mean and variance of a male character from autosomal genes in the ith parental population be μ_{iA} and σ^2_{iA} and those from X-linked genes be μ_{iX} and σ^2_{iX} for $i = 1$ and 2, we give the theoretical means and variances in the appropriate populations in Table 4. The extra genetic variance in the backcross populations, $\frac{1}{2}\sigma^2_{sA}$, is produced by the segregation of autosomal genes that differ in their average effects in the parental populations. For simplicity the environmental variance, σ^2_e, is assumed to be the same in all populations (although a linear dependence of σ^2_e on the mean values of the last three populations would not alter the following results).

Allowing for genetic variance within the parental populations, Wright's formula (14, 15) gives the effective (or minimum) number of freely segregating autosomal genes contributing to the difference between parental populations:

$$n_{EA} = (\mu_{2A} - \mu_{1A})^2/8\sigma^2_{sA}. \quad [1]$$

The effective number is always less than or equal to the actual number of genes, with equality only when all of the genes are unlinked and contribute equally to the difference between the parental populations (14, 15). There are several ways of deriving this quantity from the theoretical expressions in Table 4. However, with the present data this must be done with only the last three populations, which are not truncated by the lower limit for the expression of the character at zero cilia, and for which the mean values are nearly additive on the transformed scale of measurement. The mean difference and the segregation variance due to autosomal genes can be obtained in terms of the parameters for these three populations as

$$\mu_{2A} - \mu_{1A} = 2(\mu_{P_2} - \mu_{F_1})$$
$$\sigma^2_{sA} = 2\sigma^2_{B_2} - \sigma^2_{P_2} - \sigma^2_{F_2}.$$

Because the data are in the form of full-sib families, the autosomal segregation variance should be expressed in terms of the expected within-family variances (denoted by the additional subscript w). The use of within-family variances also helps to minimize (undetected) temporal heterogeneity in the data, since all of the families were not produced in the same generation. Under the assumption of random mating, half of the additive genetic variance and all of the environmental variance is expected to appear within full-sib families (chapter 9 in ref. 18), hence

$$\sigma^2_{sA} = 4\sigma^2_{wB_2} - 2\sigma^2_{wP_2} - 2\sigma^2_{wF_1}.$$

Therefore, an estimate of n_{EA} on the transformed scale can be derived from the data in Table 3 by using the formula

$$\hat{n}_{EA} = (\bar{\bar{x}}'_{P_2} - \bar{\bar{x}}'_{F_1})^2/8\left[\bar{s}^2_{wB_2}{}' - \frac{1}{2}(\bar{s}^2_{wP_2}{}' + \bar{s}^2_{wF_1}{}')\right], \quad [2]$$

which yields $\hat{n}_{EA} = 2.3$.

Table 4. Theoretical means and variances of a male character in crosses between populations

Population	Mean	Variance
P_1	$\mu_{1A} + \mu_{1X}$	$\sigma^2_{1A} + \sigma^2_{1X} + \sigma^2_e$
$B_1 = P_1♀ \times F_1♂$	$\frac{3}{4}\mu_{1A} + \frac{1}{4}\mu_{2A} + \mu_{1X}$	$\frac{3}{4}\sigma^2_{1A} + \frac{1}{4}\sigma^2_{2A} + \sigma^2_{1X} + \frac{1}{2}\sigma^2_{sA} + \sigma^2_e$
$F_1 = P_1♀ \times P_2♂$	$\frac{1}{2}(\mu_{1A} + \mu_{2A}) + \mu_{1X}$	$\frac{1}{2}(\sigma^2_{1A} + \sigma^2_{2A}) + \sigma^2_{1X} + \sigma^2_e$
$F_1 = P_2♀ \times P_1♂$	$\frac{1}{2}(\mu_{1A} + \mu_{2A}) + \mu_{2X}$	$\frac{1}{2}(\sigma^2_{1A} + \sigma^2_{2A}) + \sigma^2_{2X} + \sigma^2_e$
$B_2 = P_2♀ \times F_1♂$	$\frac{1}{4}\mu_{1A} + \frac{3}{4}\mu_{2A} + \mu_{2X}$	$\frac{1}{4}\sigma^2_{1A} + \frac{3}{4}\sigma^2_{2A} + \sigma^2_{2X} + \frac{1}{2}\sigma^2_{sA} + \sigma^2_e$
P_2	$\mu_{2A} + \mu_{2X}$	$\sigma^2_{2A} + \sigma^2_{2X} + \sigma^2_e$

Theoretical means and variances of a male character in parental populations, reciprocal F_1s, and backcrosses fathered by F_1 males (from either reciprocal cross) with partial sex-linked inheritance. It is assumed that there are no maternal (or cytoplasmic) effects, mating is random, and all of the genetic variance within and between populations is additive.

Studies of linked heterozygous inversions in male *D. silvestris* have not revealed any recombinants (unpublished data) suggesting that, like *D. melanogaster*, crossing-over in males is rare or absent under most conditions. The lack of recombination in males implies that the estimate of n_{EA} should be interpreted as the effective or minimum number of autosomes differentiating the races with regard to this character. *D. silvestris* has four large autosomes and one small autosome (the dot chromosome), so the maximum value for n_{EA} that could be detected by using the biometrical method is 5.

The standard error of the estimate of the effective number of autosomes is ±1.1, derived on the assumption of large samples as outlined in ref. 15. The variance of the sampling distribution of $\hat{n}_{EA}$ actually does not exist because there is a sufficiently high probability that the denominator in Eq. 2 will be near zero and the estimate will be a positive or negative number of very large magnitude. The calculated standard error nevertheless suggests the degree of confidence that can be placed in the estimate. This view was supported by constructing an empirical sampling distribution by the method of bootstrapping (19). A bootstrap distribution of $\hat{n}_{EA}$ with 2000 values was obtained by two-stage random sampling with replacement from data on the transformed scale, first sampling families and then sampling within chosen families. This gave a 90% probability that more than one autosome is involved.

Our analysis therefore indicates that genes on at least three chromosomes are involved in the differentiation of cilia numbers on the forelegs of males from the Kahuku and Kilauea populations of the bare and hairy races of *D. silvestris*. Sex-linked genes contribute about 30% of the difference, and it is likely that genes on two or more autosomes account for the remaining 70% of the difference between the races. These conclusions from F_1 and backcross data confirm our preliminary analysis based on information from F_1 and F_2 populations (20).

DISCUSSION

The evolution of the novel ciliary row on the tibia of *D. silvestris* males from the natural population at Kilauea Forest Reserve is based on genetic changes at three or more unlinked loci—at least one sex-linked and two autosomal. Not only is this population polymorphic for inversions and allozymes (21), but the data presented here show that the phenotypic variance for cilia number within populations of the Hilo-side race also has a substantial genetic component. This conclusion is supported by the results of selection experiments for high and low cilia number, carried out on laboratory stocks derived from a natural population located 6 km from Kilauea (Olaa Tract, Hawaii Volcanoes National Park) (22). A substantial response to selection was observed, indicating that there is genetic polymorphism within populations of the Hilo-side race for this character. Furthermore, as yet unidentified aspects of mating behavior in this species show both geographic (23) and intrapopulation (24, 25) genetic variability, demonstrated in mating experiments.

Lek formation, intermale competition, and female mating preferences exist in this species (12, 26, 27); accordingly we suggest that the newness, the occasional attenuation in number of individuals, and the disjunct nature of these derived populations (28) are conducive to locally occurring shifts in the polygenic basis of the premating behavioral system. This, of course, may involve numerous elements in addition to the cilia character on which we have concentrated our metrical studies.

A shift in a character, such as the one involving the embellishment of cilia number in Hilo-side males, may have been set in motion by a founder event in which a new population is started from a few individuals, producing random drift in gene frequencies. However, the origin of the novel phenotype was probably not mediated by random genetic drift alone. More likely it is the result of a realignment of the sexual selection process made necessary in the altered genetic environment produced by a founder event or random genetic drift altering the equilibrium of female mating preferences and male characters contributing to mating success.

The mode of change observed in these populations of *D. silvestris* may provide a model not only for speciation in Hawaiian *Drosophila* but also in other groups of animals characterized by a system of sexual selection based on mating preferences for polygenic characters (4).

We thank Linden T. Teramoto for painstaking care in raising the flies and mounting the legs, Craig M. Pease for assistance with the computations, and Dr. Terrence W. Lyttle for comments on the manuscript. This work was supported by National Science Foundation Grant DEB-7926692 to H.L.C. and U.S. Public Health Service Grant GM27120 to R.L.

1. Darwin, C. (1871) *The Descent of Man and Selection in Relation to Sex* (Murray, London).
2. Fisher, R. A. (1958) *The Genetical Theory of Natural Selection* (Dover, New York), Rev. Ed., pp. 135–162.
3. O'Donald, P. (1980) *Genetic Models of Sexual Selection* (Cambridge Univ., Cambridge, UK).
4. Lande, R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3721–3725.
5. Kirkpatrick, M. (1982) *Evolution* **36,** 1–12.
6. West-Eberhard, M. J. (1983) *Q. Rev. Biol.* **58,** 155–183.
7. Carson, H. L. (1978) in *Ecological Genetics: The Interface*, ed. Brussard, P. F. (Springer, New York), pp. 93–107.
8. Spieth, H. T. (1982) *Evol. Biol.* **14,** 351–437.
9. McDougall, I. & Swanson, D. A. (1972) *Bull. Geol. Soc. Am.* **83,** 3731–3738.
10. Carson, H. L., Val, F. C., Simon, C. M. & Archie, J. W. (1982) *Evolution* **36,** 132–140.
11. Craddock, E. M. (1974) *Evolution* **28,** 593–606.
12. Spieth, H. T. (1978) *Evolution* **32,** 435–451.
13. Coyne, J. A. (1983) *Evolution* **37,** 1101–1118.
14. Wright, S. (1968) *Evolution and the Genetics of Populations: Genetic and Biometric Foundations* (Univ. of Chicago, Chicago), Vol. 1.
15. Lande, R. (1981) *Genetics* **99,** 541–553.
16. Val, F. C. (1977) *Evolution* **31,** 611–629.
17. Templeton, A. R. (1977) *Evolution* **31,** 630–641.
18. Falconer, D. S. (1981) *Introduction to Quantitative Genetics* (Longman, London), 2nd Ed.
19. Efron, B. (1982) *The Jackknife, the Bootstrap and Other Resampling Plans* (Soc. Indust. Appl. Math., Philadelphia).
20. Carson, H. L. & Lande, R. (1983) *Genetics* **104,** s12 (abstr.).
21. Craddock, E. M. & Johnson, W. E. (1979) *Evolution* **33,** 137–155.
22. Carson, H. L. & Teramoto, L. T. (1984) *Proc. Natl. Acad. Sci. USA* **81,** in press.
23. Kaneshiro, K. Y. & Kurihara, J. S. (1981) *Pac. Sci.* **35,** 177–183.
24. Ahearn, J. N. (1980) *Experientia* **36,** 63–64.
25. Kaneshiro, K. Y. & Carson, H. L. (1982) *Genetics* **100,** s34 (abstr.).
26. Spiess, E. B. & Carson, H. L. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3088–3092.
27. Carson, H. L. & Teramoto, L. T. (1980) *Genetics* **94,** s14 (abstr.).
28. Carson, H. L. (1982) *Heredity* **48,** 3–25.