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SELECTION ON THE COLOR POLYMORPHISM IN HAWAIIAN HAPPY-FACE SPIDERS: EVIDENCE FROM GENETIC STRUCTURE AND TEMPORAL FLUCTUATIONS

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Abstract.—Throughout this century genetic polymorphisms for color have been widely used as a research tool to allow insights into key evolutionary processes. Although color variants can often be diverse within populations, frequencies of different morphs may be similar across populations, either as a result of balancing selection or gene flow. Under these circumstances selection can be extremely difficult to demonstrate. Here we test for balancing selection on the naturally occurring color forms of the Hawaiian happy-face spider, *Theridion grallator* with two approaches. First, allozyme loci are used to generate a null model against which to test selection. Frequencies of alleles involved in the color polymorphism of *T. grallator* are used to generate another estimate for comparison. The results suggest that statistically similar frequencies of color morphs among populations of *T. grallator* may be maintained by some form of balancing selection. Second, we make use of an unusual event in which the normally stable frequencies of unpatterned and patterned morphs within a population were found to have shifted toward an excess of unpatterned morphs. We scored offspring of all fertilized, unpatterned (bottom-recessive) females found during this period of skewed morph frequencies and also in a year when morph frequencies were normal to deduce paternal color phenotypes. Mating was found to be random in the normal year, but in the perturbed year females had mated with rare (patterned) males twice as frequently as expected on the basis of the frequency of this morph type in the population. Both of these results are consistent with selection operating on the color polymorphism, and we speculate that apostatic selection, perhaps mediated by bird predators, may provide the mechanism.

Key words.—Frequency-dependent selection, happy-face spider, population structure.

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The role that natural selection plays in promoting and maintaining variation within populations may depend on the level at which the variation is expressed (e.g., external phenotypic effects versus silent nucleotide substitutions; Cain and Provine 1991). Color polymorphisms provide an immediately tangible link between a genotype and an externally expressed phenotype, and have traditionally provided a source of variation with which to investigate selective processes. Color polymorphisms are of widespread occurrence. In many cases circumstantial evidence, such as their persistence over very long periods of time, suggests that they are maintained by natural selection. Golding (1992), for example, has argued that polymorphisms surviving speciation events, if truly homologous, are unlikely to be selectively neutral. Examples of this phenomenon include color and banding in the land snails *Cepaea nemoralis* and *C. hortensis* (Jones et al. 1977) and color pattern and spotting in the spiders *Enoplognatha ovata* and *E. latimana* (Oxford 1992), both of which predate the speciation event.

Although selection appears to operate in the maintenance of many color polymorphisms, identification of the mechanisms by which it is achieved usually depends on variation in the strength and/or direction of selection in space or time so that changes in character states can be associated with shifts in environmental variables (Brodie et al. 1995). When morph frequencies are relatively constant across populations, it becomes more difficult to differentiate between uniform selection acting over a wide area and the migration of effectively neutral alleles between populations. Under such circumstances there are two effective ways to detect selection acting to maintain frequencies in a balanced color polymorphism: (1) use of the genetic structure between populations to generate a null model against which to test for selection; and (2) examination of events within a population during periods when morph frequencies deviate from normal values. Both of these approaches are used here to investigate whether selection is implicated in the maintenance of similar color-morph frequencies in populations of an endemic Hawaiian spider, *Theridion grallator* (Araneae: Theridiidae) that exhibits extreme color polymorphism.

Several recent studies have used statistical estimates of population differentiation based on different sets of loci to assess the role of selection. If variation at one of the sets is assumed to be effectively neutral (with respect to genetic structure), significant differences between the two estimates of population structure can indicate the presence and nature of selection acting on the second set. In this way, variation at a "target" locus or loci can be compared with a null model generated from "neutral" alleles. If the neutrality assumption holds, population differentiation at the first set of loci will reflect background drift and migration. A significantly higher level of genetic structure for the second set of loci would therefore indicate a degree of differentiation greater than background and imply local, divergent selection. Conversely, a significantly lower degree of differentiation would suggest selection acting to maintain a greater uniformity of allele frequencies at the second set of loci than would be expected from the null model. This general method has been used for both polygenic characters and polymorphic loci to test for an effect of selection between subpopulations (e.g., Prout and Barker 1993; Mithen et al. 1995), and to reveal how selection operates differently on different sets of characters (e.g., Long and Singh 1995; Taylor et al. 1995).

To determine the selective mechanism acting to maintain

frequencies of color morphs within a population, we focus here on events subsequent to a departure from normal morph frequencies. In previous studies (Oxford and Gillespie 1996a,b) we have shown that the bottom recessive allele, that is, one that is recessive in expression to all other alleles, appears as unpatterned (plain Yellow, except for Red-front males on Hawaii Island) in the homozygous form. The frequency of this homozygous genotype is remarkably constant at 65–70% of the population across different populations (within and among islands) and over time (Gillespie and Tabashnik 1990). In the present study we examine color morphs in the offspring of bottom-recessive, fertilized females found during this period when morph frequencies were skewed, to ascertain the frequencies of morphs within the successful male mating set.

Methods

Study Organism and Collection Sites

The Hawaiian happy-face spider, *T. grallator*, is found on four of the larger Hawaiian Islands (Oahu, Molokai, Maui, and Hawaii). The web built by *T. grallator* is a much reduced and inconspicuous layer of silk placed on the undersides of leaves of a small proportion of the plant species available, particularly those with relatively large and entire leaves, such as *Broussaisia arguta* (Saxifragaceae) and *Clermontia arborescens* (Campanulaceae; Gon 1985). *Theridion grallator* is seasonal, with very small spiders and immatures predominating from late summer until spring; by April, most of the population consists of mature individuals (Gillespie and Tabashnik 1989). All populations exhibit a spectacular diversity of color morphs (plate 1 of Oxford and Gillespie 1996a).

Spiders were collected from the following sites: Molokai—the Nature Conservancy of Hawaii's Kamakou Preserve, elevation 1110 m; Maui—the Nature Conservancy of Hawaii's Waikamoi Preserve, Haleakala, elevation 1360 m; Hawaii—Kahua Ranch, Kohala, elevation 1152 m; Hawaii—Mauna Kea–Mauna Loa Saddle, elevation 1600 m; and Hawaii—Thurston, Kilauea, Volcanoes National Park, elevation 1190 m. Populations of *T. grallator* are patchily distributed, and at most sites occur at very low densities (in particular on Molokai). Numbers of individuals collected on Molokai were therefore limited. For the analysis of color morph frequencies over time within a population we used the East Maui Waikamoi Preserve site.

Population Differentiation and Color-Morph Frequencies

Allozymes

Allozyme frequencies were determined using cellulose acetate electrophoresis (Richardson et al. 1986). *Theridion grallator* were homogenized in water and the homogenate applied to cellulose acetate gels (Helena Laboratories, Beaumont, TX). Electrophoresis was performed at 180 V, and the gels were developed using specific enzyme detection techniques (stain recipes were adapted from Hebert and Beaton [1989], buffer recipes from Richardson et al. 1986). We used eight polymorphic enzyme systems: glyceraldehyde-3-phosphate dehydrogenase (G3PDH, E.C. 1.2.1.12), 6-phosphogluconate dehydrogenase (6PGDH, E.C. 1.1.1.44), and glycerol-3-phosphate dehydrogenase (GPDH, E.C. 1.1.1.8) (buffer for all 0.1 M tris-citrate, pH 8.2, 1 h, 1.5 h, and 0.75 h respectively); isocitrate dehydrogenase (IDH, E.C. 1.1.1.42) and mannose phosphate isomerase (MPI, E.C. 5.3.1.8) (buffer of 0.01 M citrate-phosphate, pH 6.4, 1 h); phosphoglucose isomerase (PGI, E.C. 5.3.1.9; buffer of 0.015 M tris-maleate, pH 7.2, 1 h); malate dehydrogenase (MDH, E.C. 1.1.1.37; buffer of 0.05 M tris-maleate, pH 7.2, 1 h); and phosphoglucomutase (PGM, E.C. 2.7.5.1; buffer of 0.1 M tris-maleate, pH 7.8, 1 h).

Color-Morph Alleles

On Maui (and probably Molokai) the morphs seem to be controlled by alleles at one autosomal locus and are distributed equally in both sexes. Plain Yellow (unpatterned) is bottom recessive to all patterned morphs, in which red, white, or black patches are superimposed on the yellow background (Oxford and Gillespie 1996a). On Hawaii Island, however, two pairs of morphs, all controlled by separate alleles on Maui, appear to be determined by the sex-limited expressions of two "composite" alleles. Thus, one allele is expressed as Yellow in females but Red-front in males, and the other as Red-blob in females but Red-ring in males (Oxford and Gillespie 1996b,c). Yellow in females and Red-front in males represent the bottom-recessive morphs throughout Hawaii Island. In addition, a second, unlinked, autosomal locus is indicated, although patterned morphs controlled at this locus are rare and have been found in only a single population.

We acquired estimates of allele frequencies for color morphs based on color-morph frequencies and using our knowledge of their inheritance (Oxford and Gillespie 1996a,b). Because of the differences underlying the genetics of color polymorphism on the different islands, comparison of allele frequencies based on color morphs was conducted only between populations with the same known genetic system underlying the color polymorphism, that is, within Maui Nui (Molokai and Maui) and within Hawaii Island only. The Black-ring allele at locus II on Hawaii Island is very rare (it did not occur in the surveys that formed the basis of the current study) and all other alleles act as if they are at a single locus (locus I; Oxford and Gillespie 1996c). Accordingly, for the populations and alleles scored, we assumed color-morph alleles were at a single locus on Hawaii Island as well as on Maui Nui.

Comparison of Allozymes and Color-Morph Alleles

To determine the extent of population differentiation throughout the islands we used allozyme frequencies, with *T. grallator* sampled from two populations on Maui Nui (Molokai, $n = 4$; Maui, $n = 48$), and three populations on Hawaii Island (Kohala, $n = 16$; Mauna Loa–Mauna Kea Saddle, $n = 25$; Kilauea, $n = 15$). Color allele frequencies were obtained for the same populations on Maui Nui (Molokai, $n = 60$; Maui, $n = 243$) and on Hawaii Island (Kohala, $n = 18$; Mauna Loa–Mauna Kea Saddle, $n = 28$; Kilauea, $n = 68$). Genetic exchange among populations based on allozyme markers (assumed to be neutral with respect to population structure) was determined using Weir and Cockerham's

(1984) θ, a variance component estimate of Wright's (1951) F_{ST}. These values were used to generate a null model for populations. Confidence limits for θ were obtained by bootstrapping across loci. Estimates of θ for color alleles were also made: the frequencies of the different color-morph alleles were compared to the estimates and 99.9% confidence limits based on the allozyme markers. This test of significance incorporates the *magnitude* of the difference between θ estimated from allozymes and θ estimated from color morphs.

Because of the sparse data from Molokai, an additional test was used for both Maui Nui and Hawaii Island populations that accommodates very small sample sizes. Chi-squared analyses of allozyme counts between populations were used to assess the degree of population differentiation (heterogeneity). For comparison, heterogeneity chi-squared values were also estimated on the basis of the color alleles. Because expected class sizes were small for both allozyme and color-morph alleles, we used a Monte Carlo method to test the significance of the chi-squared values observed (Roff and Bentzen 1989). This method allows the data to be examined without combining alleles. A distribution of chi-squared values is generated by randomizing (in this case 1000 times) the data subject to the constraints that row and column totals remain fixed. The probability of obtaining a chi-squared value equal to, or greater than, the figure actually observed is determined directly from this distribution: the standard error of this probability is given by $\sqrt{(P[1 - P]/N)}$ (Roff and Bentzen 1989).

Analysis of Color-Morph Frequencies within a Population

Temporal Variation in Morph Frequencies

For the purposes of this part of the study we combined all patterned morphs as a single category because patterned morphs are individually generally at very low frequency. Seasonal fluctuations in morph frequencies were determined for one year in the Waikamoi Preserve population by monitoring the site continually from September 1987 until July 1988, and making monthly estimates of morph frequencies from all individuals found. Evidence for differences in morph frequencies between mature males and females was also checked. The proportion of unpatterned to patterned morphs was determined in the middle of each month. Yearly fluctuations in morph frequencies were determined from one-day sampling periods between April and July in 1988, 1990, 1991, 1993, and 1994. For between-year comparisons we focused on 1988 and 1993, the two years from which the largest samples were obtained and in which morph frequencies of the offspring of all females were determined.

Reproductive Response to Departures from Normal Frequencies

Within the Waikamoi Preserve population we examined the progeny of unpatterned (bottom-recessive) females in April 1988 (the month in which most of the population had reached maturity), when unpatterned represented 68% of the population, and in July 1993, when it represented 84%, to ascertain the proportion that had mated with patterned or unpatterned males. The frequency of the unpatterned morph in 1988 was typical of frequencies found in all other samples except that of 1993. In 1988, mature females were enclosed with their egg sacs in cheese-cloth bags in the field (Gillespie and Tabashnik 1989). Color morphs of the offspring were scored at various stages as different patterns became established at different rates. In 1993, mature females were isolated in the laboratory in 300 mL plastic cups sealed with a lid of nylon mesh and maintained in an incubator at 16–18°C with a light cycle of 12:12 L:D (Oxford and Gillespie 1996a). Most of these individuals had been inseminated in the wild and laid egg sacs within two weeks of capture. Color morphs of the offspring were scored as in 1988.

Seasonal Changes in the Movement of Individuals

Spiders may suffer a reduction in crypticity when they move around (Avery and Krebs 1984). Because of this, we estimated movement in *T. grallator* in the Waikamoi Preserve population by trapping spiders arriving on leaves over three-day periods throughout the year. For each of the three predominant plant species used by *T. grallator* (*Broussaisia, Clermontia*, and *Hedychium*), we coated the undersides of six leaves with a nondrying, sticky tree-banding compound. We removed and recorded individual *T. grallator* trapped on these leaves every third day from September 1987 to August 1988. When sticky leaves senesced, we used the leaf closest to them instead.

Results

Population Differentiation and Color-Morph Frequencies

Allele frequencies based on the allozymes and color-morph alleles are given in Table 1. We tested each of the allozyme loci for conformation to Hardy-Weinberg proportions using Levene's (1949) correction for small sample size in BIOSYS-1 (Swofford and Selander 1981). We adjusted the probability per comparison using the Dunn-Sidák method (Sokal and Rohlf 1995, p. 702), and found that the only locus to show any significant deviation from the Hardy Weinberg equilibrium was GPDH2 on Maui. Accordingly, this locus was not considered in any further calculations involving Maui Nui populations.

Comparison of Allozymes and Color-Morph Alleles

Because calculations of θ assume that selection does not influence the polymorphism, that is, gene flow is the only factor restricting differentiation among subpopulations, the role of selection on the color locus can be assessed by contrasting values generated using frequencies of color-morph alleles with those derived from allozymes. Local selection on the color polymorphism in different directions in different populations would give higher θ-values, whereas global selection on color in the same direction would give lower θ-values, compared with those generated using allozyme data. The estimate of population subdivision based on color-morph alleles was much lower than the value based on allozymes for the same three populations on Hawaii Island, and the difference was highly significant (Table 2). However, on Maui Nui, although the color allele θ-value was an order of mag-

TABLE 1. Frequencies of allozymes in five populations of *T. grallator*. * = did occur, but in less than 0.05% of population. † = allelic combinations on Hawaii Island only (Oxford and Gillespie 1996b).

Locus	Molokai	Maui	Kohala	Saddle	Kilauea
6PGDH (*N*)	4	48	16	24	15
A	0.00	0.02	0.00	0.02	0.03
B	1.00	0.95	0.84	0.98	0.93
C	0.00	0.02	0.16	0.00	0.03
D	0.00	0.01	0.00	0.00	0.00
MDH-1 (*N*)	4	40	16	21	10
A	0.13	0.14	0.25	0.48	0.50
B	0.88	0.43	0.75	0.48	0.45
C	0.00	0.36	0.00	0.05	0.05
D	0.00	0.08	0.00	0.00	0.00
IDH-1 (*N*)	4	48	16	23	15
A	1.00	0.41	0.09	0.46	0.17
B	0.00	0.20	0.91	0.54	0.63
C	0.00	0.40	0.00	0.00	0.20
PGM-1 (*N*)	4	8	12	17	8
A	0.38	0.28	0.42	0.50	0.37
B	0.00	0.00	0.13	0.12	0.25
C	0.63	0.67	0.46	0.38	0.38
D	0.00	0.04	0.00	0.00	0.00
E	0.00	0.01	0.00	0.00	0.00
PGI-1 (*N*)	4	40	15	25	12
A	0.00	0.00	0.00	0.00	0.13
B	0.00	0.05	0.70	0.98	0.79
C	0.63	0.19	0.10	0.00	0.00
D	0.00	0.01	0.00	0.00	0.00
E	0.37	0.65	0.20	0.00	0.04
F	0.00	0.05	0.00	0.02	0.04
G	0.00	0.03	0.00	0.00	0.00
H	0.00	0.03	0.00	0.00	0.00
GPDH1 (*N*)	4	48	16	24	13
A	1.00	0.86	0.22	0.48	0.31
B	0.00	0.14	0.78	0.52	0.69
GPDH2 (*N*)	2	5	10	13	4
A	0.50	0.50	0.00	0.54	0.25
B	0.50	0.50	1.00	0.46	0.75
MPI-1 (*N*)	4	43	16	23	12
A	0.00	0.00	0.06	0.20	0.17
B	0.00	0.01	0.00	0.02	0.04
C	0.13	0.17	0.06	0.00	0.00
D	0.00	0.00	0.00	0.04	0.00
E	0.50	0.48	0.03	0.39	0.25
F	0.37	0.21	0.69	0.33	0.54
G	0.00	0.01	0.09	0.02	0.00
H	0.00	0.12	0.06	0.00	0.00
Color (*N*)	60	243	18	28	68
Yellow	0.83	0.84	—	—	—
Yell./Red-fr†	—	—	0.90	0.91	0.86
Red-front	0.10	0.05	—	—	—
Red-front and -back	0.00	0.02	0.07	0.05	0.08
Red-lines	0.03	0.02	0.01	0.02	0.00
Red-ring	0.03	0.02	—	—	—
Red-ring/blob†	—	—	0.02	0.00	0.06
Black-ring	0.00	0.02	0.01	0.02	0.00
Black-blob	0.00	0.00*	0.00	0.00	0.00
Red-blob	0.01	0.01	—	—	—
Red-back	0.00	0.00*	0.00	0.00	0.00
White	0.00	0.01	0.00	0.00	0.00

nitude lower than that for allozymes (and comparable to the differences on Hawaii Island), the small sample size for allozyme frequencies on Molokai ($n = 4$) precluded the generation of meaningful confidence intervals and hence a test of significance.

Because of the small allozyme sample size on Molokai we also used chi-squared tests to determine the presence of genetic structuring based on the two datasets. Using the randomization procedure of Roff and Bentzen (1989), we generated eight (seven on Maui Nui) independent estimates of probability for the allozymes. The probability estimates across loci within islands were combined (Sokal and Rohlf 1995, p. 795) to give an overall indication of the degree of genetic differentiation. For the color alleles, we assumed that there was a single locus with multiple alleles in each of the island systems (Maui Nui and Hawaii Island). The results (Table 3) showed that there was significant overall genetic differences between populations based on allozyme frequencies, both within Maui Nui and within Hawaii Island. In contrast, analyses of the color alleles did not indicate significant differences between populations. The limits of the chi-squared procedure should be noted. In particular, it cannot quantify genetic differentiation between populations, but merely indicates its presence. The sample of allozyme loci allows a test of genetic structure between populations based on a set of loci on which selection was either minimal or at least did not correspond at all loci to the variation in color pattern. To provide such a test, a single allozyme locus would be insufficient. Using multiple loci, significant differentiation was detected. The color locus, however, is the only locus involved in the trait of interest. The results indicate no significant genetic differentiation at the color locus. However, this test lacks the power of the bootstrapping analysis, and our failure to reject the null hypothesis (of no differentiation) does not, of course, prove that differences are absent. However, the fact that the results of both analyses are in agreement lends support to the argument that on Maui Nui as well as on Hawaii Island the color alleles are more similar between populations than would be expected on the basis of the differentiation at neutral electrophoretic loci.

Analysis of Color-Morph Frequencies within a Population

Temporal Variation in Morph Frequencies

The frequency of unpatterned:patterned morphs remained almost constant through the year (September 1987 until July 1988), varying only between 61% and 71% unpatterned across months. There was no evidence of heterogeneity between months ($G_H = 3.47$, df = 7, $P = 0.839$; Fig. 1). Note that although populations were sampled independently each month, it is possible that some of the same individuals were scored in consecutive months. Undoubtedly there was some turnover during the sampling period, and, despite this, morph frequencies remained stable. Males, which matured between March and May, were found to exhibit the same morph types as females and showed no significant difference in morph frequency as compared to females found in the same period (see below).

Between years, morph frequencies remained fairly constant

TABLE 2. Comparison of values of population subdivision (θ) for two Maui Nui populations (Molokai and Maui) and three populations on Hawaii Island (Kohala, Mauna Loa–Mauna Kea Saddle, and Kilauea) calculated from frequencies of: (A) eight neutral allozyme loci; (B) color alleles. Frequencies of color alleles were obtained using dominance relationships based on breeding studies to estimate allele frequencies (Gillespie and Tabashnik 1990; Oxford and Gillespie 1996a,b).

	Population differentiation ($\theta \pm$ 99.9% CL)	
Estimates based on:	Maui	Hawaii Island
A. Allozymes (99.9% CL)	0.067	0.108
	—	(0.014–0.258)
B. Color-morph alleles	0.008	0.011

except for 1993, when the proportion of unpatterned morphs was much higher (Fig. 2). Comparing all years there was no significant heterogeneity in the proportion of unpatterned morphs ($G_H = 7.90$, df = 4, $P = 0.095$). However, frequencies of unpatterned morphs were significantly higher in 1993 as compared to 1988, the years in which females were progeny tested ($G_H = 6.78$, df = 1, $P = 0.009$). Because seasonal variation showed no significant heterogeneity, the annual samples are unlikely to vary as a result of them having been taken at slightly different times of year.

Reproductive Response to Departures from Normal Frequencies

Bottom-recessive (unpatterned) females in a population will sample reproductively active males. Segregation ratios of patterned to unpatterned morphs in the progeny of wild-inseminated females suggest that multiple mating, if it occurs at all, is rare (Gillespie and Tabashnik 1989; Oxford and Gillespie 1996a,b). Accordingly, we can use the progeny of the females to compare the frequencies of unpatterned and patterned sires with the frequencies of these morphs in the general population. To determine sires we used only broods containing at least five progeny, a sample size that reduces the probability of mistaking a brood segregating 1:1 for unpatterned:patterned for one containing all unpatterned individuals to less than 0.05. The 1988 population sample contained 91 unpatterned (47 females, 44 males) and 43 patterned (23 females, 20 males) individuals, with no significant differences in morph frequencies between the sexes ($G_{adj} = 0.039$, df = 1, Williams' correction; Sokal and Rohlf 1995). The overall proportion of the unpatterned morph was 0.68. The cohort of 17 unpatterned, wild-mated females had mated with 11 unpatterned and six patterned males. The difference between morph frequencies in males sampled by the females and in our total population sample is not significant ($G_{adj} = 0.067$, df = 1). In 1993 we sampled 63 unpatterned (57 females, 6 males) and 12 patterned (all female) individuals (overall proportion of unpatterned morph 0.84). The differences in morph frequencies between the sexes are not significant (Fisher's exact test, $P = 0.581$). Of the 31 unpatterned females whose broods were examined, 19 had mated with unpatterned males and 12 with patterned males. There is a significant difference in the morph frequencies of males, as sampled by females, and our total population sample ($G_{adj} = 5.902$, df = 1, $0.02 > P > 0.01$). Females were mating with patterned males with a frequency of 0.39 (12/31), whereas we sampled patterned morphs in the population with a frequency of only 0.16 (12/75). As mentioned above, multiple

TABLE 3. Comparison of chi-squared estimates of population subdivision for two Maui Nui populations (Molokai and Maui) and three populations on Hawaii Island (Kohala, Mauna Loa–Mauna Kea Saddle, and Kilauea) calculated from frequencies of: (A) eight neutral allozyme loci (seven on Maui Nui); 1 = 6PGDH; 2 = MDH; 3 = IDH; 4 = PGM; 5 = PGI; 6 = GPDH1; 7 = GPDH2; 8 = MPI; (B) color alleles. Frequencies of color alleles were obtained using dominance relationships based on breeding studies to estimate allele frequencies (Gillespie and Tabashnik 1990; Oxford and Gillespie 1996a,b). The randomization method of Roff and Bentzen (1989) was used to generate a distribution of 1000 chi-squared values for each locus from which the probability (P) of obtaining the observed chi-squared values can be judged (see text for details).

Island	Alleles	Locus	# Alleles (df)	Observed χ^2	P (2SEs)
Maui Nui (2 pops.)	Allozymes	1	3 (2)	0.351	0.985 (0.0077)
		2	3 (2)	6.669	0.024 (0.0097)
		3	3 (2)	10.511	0.003 (0.0034)
		4	2 (1)	0.638	0.770 (0.0266)
		5	5 (4)	7.585	0.083 (0.0174)
		6	2 (1)	1.238	0.058 (0.0147)
		8	8 (7)	2.129	0.670 (0.0297)
				$-2\Sigma\ln P = 31.104$, df = 14, $0.001 < P < 0.01$	
	Color alleles	1	10 (9)	14.432	0.130 (0.0213)
Hawaii Island (3 pops.)	Allozymes	1	2 (2)	10.295	0.024 (0.0097)
		2	3 (4)	7.424	0.098 (0.0188)
		3	3 (4)	30.240	< 0.001* (0.0020)
		4	3 (4)	2.112	0.686 (0.0293)
		5	4 (6)	31.922	< 0.001* (0.0020)
		6	2 (2)	6.048	0.039 (0.0122)
		7	2 (2)	19.031	< 0.001* (0.0020)
		8	6 (10)	33.685	< 0.001* (0.0020)
				$-2\Sigma\ln P = 74.609$, df = 16, $P \ll 0.001$	
	Color alleles	1	5 (8)	6.794	0.542 (0.0315)

* Assumed to equal 0.001 for SE and combining probabilities.

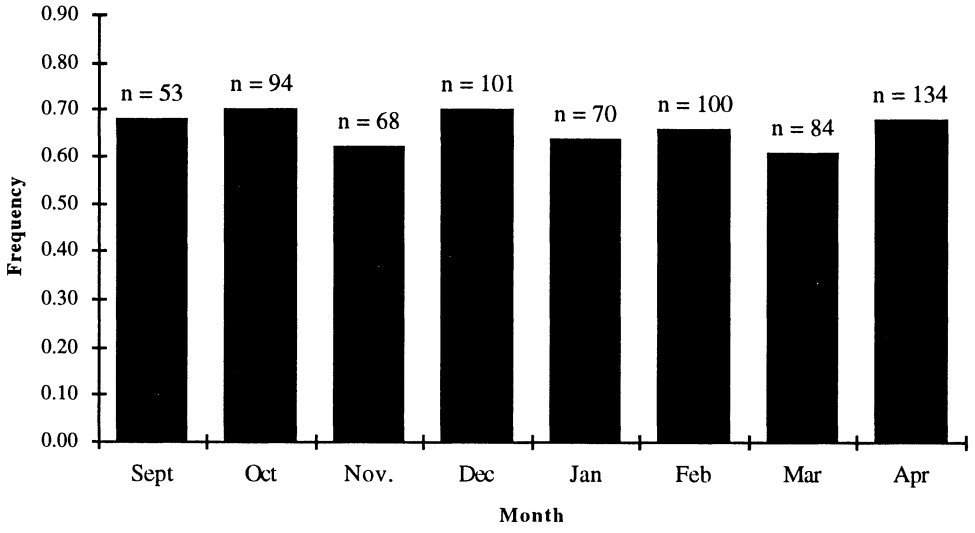

FIG. 1. Seasonal fluctuations in the frequency of the most common morph (unpatterned) at Waikamoi, Maui, 1987–1988.

mating is rare or absent in *T. grallator*, and it is extremely unlikely, therefore, that multiple matings will have affected our estimates of morph frequencies in reproductively active males. We also have no reason to believe that the different methods of rearing broods (enclosed bags in the field vs. laboratory conditions) in the two years will affect our ability to discriminate progeny sired by patterned or unpatterned males.

Seasonal Changes in the Movement of Individuals

Very few *T. grallator* were caught on the leaves through most of the year, and these were almost all immatures (Fig. 3). However, the numbers caught (and hence movement) increased significantly between mid-March and mid-June. During this time, the individuals caught were all mature males.

DISCUSSION

The establishment and maintenance of genetic variation has puzzled scientists for over a century. Recognition of the phenomenon as visible polymorphisms, particularly those relating to color, led to some of the first investigations into the genetics of natural populations (e.g., Gulick 1872). Despite the long history of investigation, evidence for any selective value of color polymorphisms has often been elusive. One of the most intensively studied color polymorphisms is that of *Cepaea* land snails, in which morph frequencies appear to be influenced by a number of factors including visual selection by predation (Cain and Sheppard 1954), climatic selection (Jones et al. 1977), sampling drift (Wright 1978), and founder/bottleneck effects (Cameron and Dillon 1984). A similar array of factors have been implicated in the main-

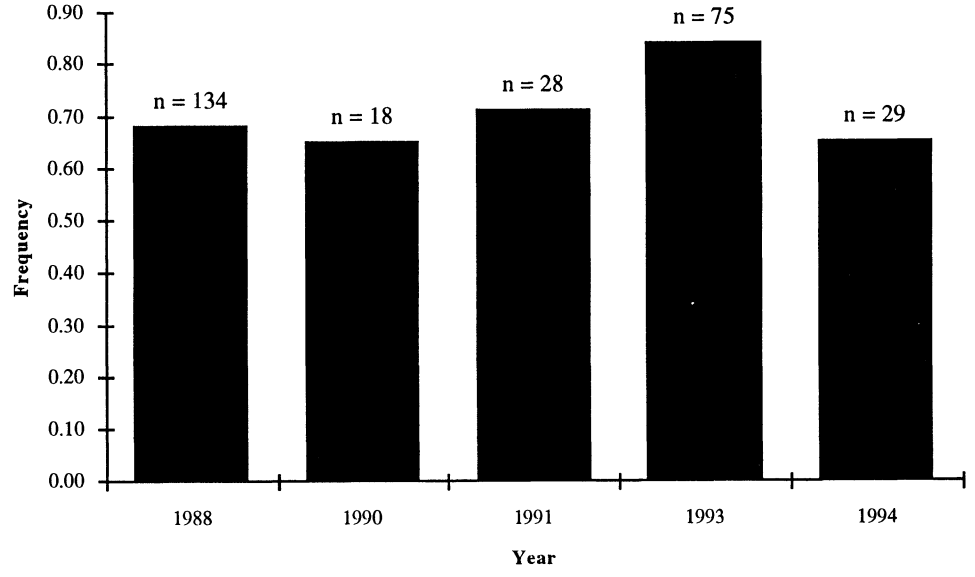

FIG. 2. Yearly fluctuations in the frequency of the most common morph (unpatterned) at Waikamoi, Maui, in different years.

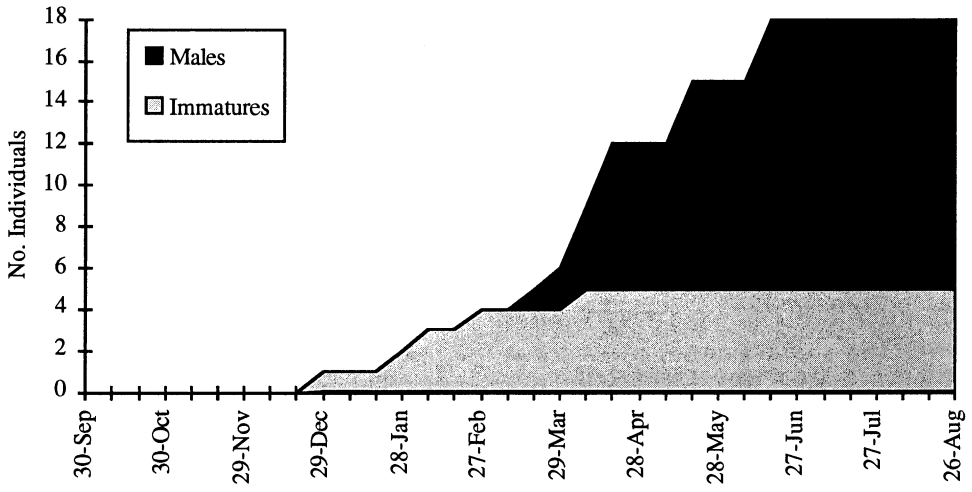

FIG. 3. Cumulative number of individual *Theridion grallator* caught on sticky leaves, September 1987 to August 1988.

tenance of color polymorphisms in other taxa. For example, opposing effects of selection for crypticity and gene flow may be responsible for the variability in pattern of Lake Erie island water snakes (King 1992; King and Lawson 1995). Among spiders, the color polymorphism of *Enoplognatha ovata* (Theridiidae) ultimately appears to be maintained by balancing selection, but with genetic drift dictating morph frequencies in local populations most of the time (Oxford and Shaw 1986; Oxford and Reillo 1993). Gunnarsson (1985) examined another spider, *Pityohyphantes phrygianus* (Linyphiidae), and showed that morph frequencies are balanced by opposing selective pressures: melanic forms have an activity advantage over nonmelanics at low temperatures, but at the same time this renders them more vulnerable to predation.

In *T. grallator* the relative frequencies of unpatterned and patterned color morphs (as defined above) are remarkably constant in all populations examined here, despite variation in climate (primarily due to elevational differences at different sites) and habitat (due to isolation of the islands). The current results, taken overall, suggest that selection is responsible for the maintenance of the color polymorphism both between populations and within populations over time. This conclusion stems from two lines of evidence. First, between-population analyses showed that, while neutral loci (allozymes) demonstrated significant population structuring, alleles at the color locus indicated little or none. We used a duel approaches to the detection of population substructuring for the two sets of loci examined: estimations of θ and their comparison by bootstrapping and chi-squared tests to detect heterogeneity in allele counts among populations. Both analyses suggest that the degree of population differentiation is much less for color alleles than for allozymes. Second, comparisons between morph frequencies in the population and those in reproductively successful males indicated that, in a year when the frequency of the unpatterned morph was higher than normal, significantly more patterned males than expected sired offspring. In contrast, in a year when morph frequencies were much more typical, mating success of patterned males reflected their frequency in the population as a whole. The consequence of patterned males having a mating advantage when this morph is relatively rare in the population will be the return of morph frequencies toward the apparently stable state (negative frequency dependence). Both of these lines of evidence suggest a role for selection: the first implies that some sort of balancing selection is acting in a similar way across different populations, and the second indicates what form the selection may take.

There are several mechanisms by which frequency-dependent selection might be achieved. For example, it is known to occur through the action of differential selection in a heterogeneous environment with gene flow acting to prevent complete differentiation and loss of polymorphism (Levene 1953; Arnold and Anderson 1983; King and Lawson 1995). The heterogeneity may be due to differences in substrate and hence crypsis (Wright 1978; Sandoval 1994) or climate (Richardson 1974; Jones et al. 1977; Berry and Willmer 1986; Cowie 1990), sometimes coupled with differences in habitat selection in different morphs (Johnson 1981; Halkka and Halkka 1990). However, in the *T. grallator* system there appears to be little gene flow between populations, as evidenced by the relatively high θ-values between populations (Table 2). A second explanation is that frequency-dependent mating success may be important in maintaining color polymorphisms as has been found, for example, in the Two-Spot Ladybird *Adalia bipunctata* (summarized by Majerus 1994). In *T. grallator* the greater number of fertilizations by patterned than by unpatterned males when population morph frequencies are skewed toward the unpatterned morph might be mediated by female choice. This rare-male advantage phenomenon has been discussed extensively for a number of arthropods (Knoppien 1985; Salceda and Anderson 1988; Partridge 1989). However, most of these studies concern well-sighted insects in which courtship displays are highly visual. With the notable exception of a small number of families, most spiders, including the Theridiidae, have poorly developed visual acuity (Land 1985). It is unlikely therefore that coloration plays a direct role during mate selection in this species. However, the possibility exists of pleiotropic interactions among color morphs, courtship behavior, and female

choice, and a role for sexual selection cannot be dismissed entirely.

Another mechanism widely implicated in the frequency-dependent maintenance of visible polymorphisms involves the searching behavior of predators. Clarke (1969) and others (reviewed in Allen 1988) have argued that frequency-dependent selection on color morphs as a result of differential predation (apostatic selection) is responsible for the maintenance of many visible polymorphisms. Experiments have shown that, for example, the equilibrium frequency of morphs to which the system tends need not be the same for all morphs, and, in particular, cryptic prey maintain their protection at higher frequencies than noncryptic prey (Clarke 1962). If morph frequencies of such a system are to remain stable over time, it must also follow that relatively small shifts away from the equilibrium will result in a selective return. Despite all the interest, there are still very few cases in which apostatic selection on color morphs has actually been demonstrated under natural conditions. Perhaps the best example is provided by Reid (1987), who investigated the loss of color morphs from manipulated populations of the polymorphic mangrove snail *Littoraria filosa*.

In the *T. grallator* system, we speculate that apostatic selection might be responsible for the similarity in color morphs between populations. Selection is most likely mediated by sight-hunting avian predators that are known to feed on spiders in Hawaii (Perkins 1913). Indeed, selection for crypsis appears to have played a particularly important role in the evolution of *T. grallator* (Oxford and Gillespie 1998). Its coloration and flattened position make it highly cryptic in the green light transmitted through the leaf (Gon 1985). Significantly, *T. grallator* reverts from diurnal immobility to genus-specific postures during nocturnal activity, suggesting that the colors and other adaptations are for the avoidance of visually hunting, day-active predators. The translucent yellow (unpatterned) morph, which appears to be the most cryptic, is invariably the most frequent in populations, as expected under apostatic selection models.

The importance of visually hunting predators in the maintenance of the color polymorphism is supported by the second part of the study, as the greater number of fertilizations achieved by patterned males during the period of higher-than-normal frequencies of the unpatterned morph could be explained by any mechanism that causes decoupling of the selective pressure on males and females. This could occur during a phase in the life cycle when the sexes are differentially active and/or occupy dissimilar habitats. As we have suggested above, the most likely selective agent in the color polymorphism of *T. grallator* is predatory birds, which will be searching for the most conspicuous prey. Moving prey are almost inevitably more conspicuous and therefore more vulnerable than sedentary prey, as has been shown for spiders by Avery and Krebs (1984). Because the movement of *T. grallator* is heavily biased toward males in the reproductive season, selection as a result of predation would be expected to act more strongly on males. Therefore, when the frequency of unpatterned morphs becomes excessively high, bird predators may develop a search image for this morph and would select more strongly against it among the vagile males. This would mean that the pool of males eventually reaching, and mating with, the females would be enriched in the rarer morph. In this way, rare males can achieve a reproductive selective advantage without any involvement of female choice.

In conclusion, we provide evidence for the role of some form of balancing selection in generating similar morph frequencies within and among populations of *T. grallator* and speculate that apostatic selection may be the mechanism by which the color polymorphism is maintained. Clearly, further work is required to substantiate these suggestions.

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