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ECOLOGICAL GENETICS OF ABDOMINAL PIGMENTATION IN *DROSOPHILA FALLENI*: A PLEIOTROPIC LINK TO NEMATODE PARASITISM

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Abstract.—*Drosophila falleni* belongs to the *quinaria* species group, whose species vary considerably in patterns of wing and abdominal pigmentation. *Drosophila falleni* itself exhibits substantial variation among wild flies in abdominal spotting patterns. A selection experiment revealed that natural populations of *D. falleni* harbor high levels of genetic variation for spot number; in 10 generations of selection modal spot number within populations declined from 18 (the modal number in wild-caught females) to as low as zero. Rearing flies at different temperatures shows that some of the variation among wild flies is likely to reflect variation in the environmental conditions under which they developed. Fitness assays did not reveal any cost of reduced spot number with respect to development time, adult survival, or female fecundity. However, spotless flies were almost twice as susceptible to infection by the nematode parasite *Howardula aoronymphium*. Thus, selection exerted by nematode parasites may influence pigmentation patterns and other, genetically correlated traits in natural populations *D. falleni*.

Key words.—Abnormal abdomen, genetic variation, host-parasite interactions, interspecific variation, intraspecific variation, nematode parasitism.

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Fossil evidence and comparisons among extant lineages show that rates of morphological evolution vary dramatically through time, between characters, and among lineages (Simpson 1942; Cherry et al. 1978; Gingerich 1983; MacFadden 1988). Directional or stabilizing selection in response to environmental conditions is a likely cause of rate heterogeneity, but other proposed hypotheses include developmental constraints (Alberch 1982), shifting-balance peak shifts (Wright 1977), invasion of new adaptive zones or geographical regions (Simpson 1942), and the independence of diverging populations conferred by reproductive isolation between them (Futuyma 1987). Although poorly represented in the fossil record, the genus *Drosophila* recapitulates the pattern of evolutionary rate heterogeneity on a small scale, as species groups within the genus vary substantially in the extent to which their constituent species have diverged morphologically.

Heterogeneity among species groups of *Drosophila* in rates of morphological evolution is clearly evident for pigmentation patterns. For example, species within the *obscura* group are very similar in their abdominal pigmentation, which is characterized by a nearly uniform dark brown color. The *melanogaster* subgroup is, except for *D. santomea* (Llopart et al. 2002), also characterized by near uniformity among species in external morphology, but these species do exhibit sexual dimorphism in abdominal pigmentation (Kopp et al. 2000). In marked contrast, the picture-winged *Drosophila* of Hawaii (Spieth 1982) and the *dunni* subgroup (*cardini* group) species inhabiting Caribbean islands (Heed and Krishnamurthy 1959) exhibit substantial variation in pigmentation among species.

The genetics of interspecific differences in color pattern have been examined in several cases, including *D. virilis*-*D. novamexicana* (Spicer 1991), *D. santomea*-*D. yakuba* (Llopart et al. 2002), and the *D. dunni* complex (Hollocher 2000; Hollocher et al. 2000). In each case, several loci contribute to the differences between species, although particular chromosomal regions may have disproportionately large effects. Candidate gene sites have shown that species-specific patterns of pigmentation have been found to be correlated with differences in the expression of the *yellow*, *ebony,* and loci *bric-a-brac* genes (Wittkopp et al. 2002a,b, 2003a; Gompel and Carroll 2003).

Because differences between species must initially occur as intraspecific variation within at least one species, the genetics of *intraspecific* variation can provide information on the origin of species-level differences. In *D. melanogaster*, mutations in several genes—including *bric-a-brac*, *yellow*, *ebony*, *optomotor blind*, *Abdominal B*, and *Dopa decarboxylase*—affect pigmentation (True et al. 1999; Hollocher et al. 2000; Kopp et al. 2000). Until recently, however, there has been little data on the extent to which variation at these or other loci affect pigmentation in natural populations. To this end, Kopp et al. (2003) examined variation in abdominal pigmentation among recombinant inbred lines derived from two wild-caught flies, and found that much of the variation is determined by a single QTL allelic to the *bric-a-brac* gene. Outside of *D. melanogaster*, intraspecific variation for pigmentation has been documented in several other species of *Drosophila*, but few studies have quantified levels of variation in natural populations (reviewed in Wittkopp et al. 2003b).

To understand how pigmentation differences among species arise, one must know not only the genetic and developmental basis for the different pigmentation patterns, but also how population-level processes, especially natural selection, govern the dynamics of these polymorphisms. In other organisms, patterns of pigmentation provide some of the best examples of natural selection being of adaptive significance with respect to crypsis, aposematism, mimicry, thermoregulation, UV protection, and social signaling (Majerus 1998; Cloudsley-Thompson 1999).

Although *Drosophila* is a classic model organism, and there is growing interest in the evolution and development of pig-

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mentation patterns, very little is known about the adaptive significance of these patterns within or among species of *Drosophila* (Wittkopp et al. 2003b). For instance, Spicer (1991) speculated that the light color of *D. novamexicana*, which belongs to the largely dark-colored cool-climate-inhabiting *virilis* group, is adaptive to desert conditions. Species or subspecies of the *D. dunni* subgroup from Puerto Rico and the northern Lesser Antilles have relatively lightly pigmented abdomens, whereas those of the more southern Lesser Antilles are darker (Heed and Krishnamurthy 1959). Hollocher et al. (2000) show that this cline in pigmentation is better correlated with latitude than with fly phylogeny, suggesting that differences in pigmentation result from natural selection, perhaps mediated via climatic differences among the islands. Templeton et al. (1989) found life-history differences between *abnormal abdomen* and wild-type individuals of *D. mercatorum*, with the former having slower egg-to-adult development and reduced adult longevity, but greater early adult fecundity. These authors suggest that environmental heterogeneity, favoring different life histories, could maintain the *abnormal abdomen* polymorphism.

Here we examine variation and selection on abdominal pigmentation in a species belonging to the *Drosophila quinaria* group. The *quinaria* group, which has a Holarctic distribution, is relatively young, but speciose, indicating that it is undergoing rapid diversification (Perlman et al. 2002). Its species differ in a number of ways, including breeding site, geographical range, resistance to mushroom toxins (Spicer and Jaenike 1996), infection with *Wolbachia* (Werren and Jaenike 1995), and susceptibility to various species of nematode parasites (Perlman and Jaenike 2003). In terms of external morphology, there is considerable variation among species in both wing and abdominal spot patterns, which vary independently among species (Fig. 1). Our focus in this paper is on one member of this group, *D. falleni*.

Drosophila falleni is one of the less heavily pigmented species in this group (Fig. 1) and exhibits considerable variation among flies collected from nature, with the number of abdominal spots ranging from zero to 20. In this paper, we explore the genetic and environmental contributions to this variation and assess the fitness effects of spot number variation. The results show that there is abundant genetic variation for spot number variation within natural populations of *D. falleni*. We also show that spotless flies differ from more heavily spotted flies in their resistance to nematode parasitism, but not in other components of fitness. Thus, patterns of abdominal pigmentation and resistance to parasite infection may evolve as correlated responses to selection in natural populations of *D. falleni*.

Materials and Methods

Wild Flies

Drosophila falleni were collected from two areas about 15 km apart in the vicinity of Rochester, New York (Mendon Ponds Park and Cobbs Hill Park). Collections were made from 8–12 July 1996 and 21–23 June 1997. Spot numbers were determined for about 200 males and 200 females at each site in each year. Although the flies used in these studies were from the Rochester area, they are likely to be representative of *D. falleni* as a whole, as this species exhibits extremely low levels of differentiation among populations in the northeastern United States at both nuclear (mean $F_{ST} = 0.004$ for six allozyme loci) and mitochondrial ($\Phi_{ST} = 0.008$) markers (Shoemaker and Jaenike 1997).

Selection for Reduced Spot Number

The goal of this experiment was to determine whether there is genetic variation for spot number in *D. falleni*, and the extent to which this phenotype might be changed as a result of selection. The flies used to start the selection experiments were collected as adults in July 1996 from Cobbs Hill and Mendon Ponds Park. Each population was started with 20 females and 15 males. Nineteen populations were set up: six control and 13 experimental. Each population was maintained at 20.5°C in Mason jars on medium consisting of Instant Drosophila Medium (Carolina Biological Supply Burlington, NC) plus commercially available *Agaricus bisporus* mushrooms. Each generation, about 300 emergent flies per population were aged for one week prior to establishment of the next generation. For the experimental populations, the 20 females and 20 males with the fewest spots were selected as parents for the next generation. Because the number of spots per fly was approximately normally distributed (see Fig. 3 below), this corresponds, at least in the early generations, to an intensity of selection (i) of about 1.6 (Falconer 1981). Control populations were maintained with 20 females and 20 males randomly chosen as parents each generation, so that the control and selected populations would experience similar levels of inbreeding at loci not linked to those affecting spot number. After 10 generations of selection, 150–200 flies from each population were scored for spot number.

The six control populations and 11 of the 13 experimental populations were maintained under relaxed selection from generations 11 through 25. Populations were maintained with the same numbers of flies as previously, but flies were chosen randomly with respect to spot number. At generation 25, a sample of 150–200 flies per population was again scored for spot number.

Effect of Rearing Temperature

Flies that differed in spot number were obtained from six different strains that had been maintained in the laboratory at 20.5°C and that differed substantially in spot number. Three categories of flies were used: wild-type (males with 16 spots, females with 18–20 spots), spotless (no spots in either males or females), and intermediate (males with 4–14 spots, and females with 6–10 spots). The wild-type and spotless flies were obtained from our control and selected lines of flies (as described below), respectively, whereas the intermediate flies were obtained from both lines. A total of 150 single-pair crosses was set up, with 50 each of wild-type × wild-type, spotless × spotless, and intermediate × intermediate. Females were kept at 20°C and allowed to oviposit in three vials in successive 24-h periods. The cultures contained Carolina Instant Drosophila Medium plus a piece of commercial *Agaricus bisporus* mushroom. The three vials were randomly assigned to one of three temperature treatments for rearing the offspring: 16°C, 20.5°C, and 26°C. Upon

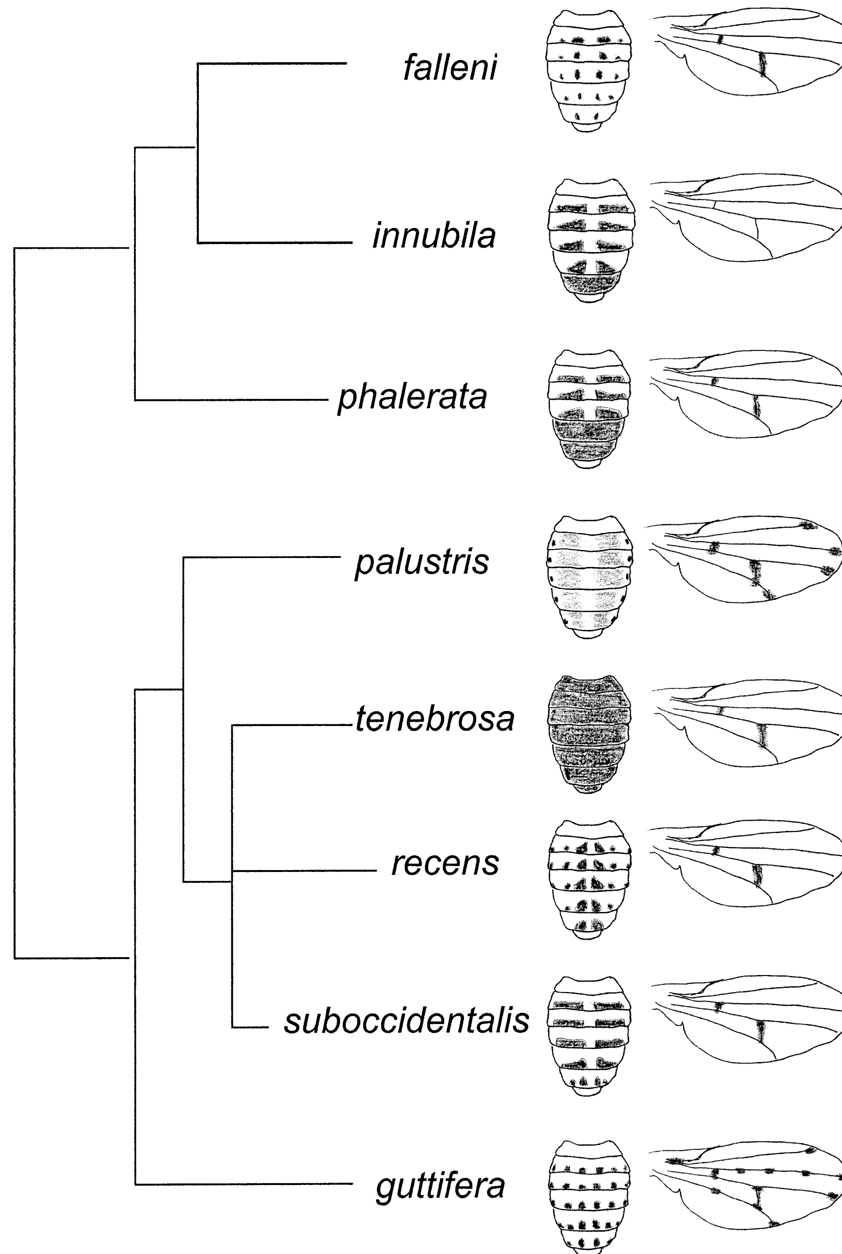

FIG. 1. Phylogenetic relationships of selected *Drosophila quinaria* group species, showing typical wing and abdominal spotting patterns. The phylogeny was inferred from mitochondrial COI, II, and III DNA sequences (Perlman et al. 2002; S. Perlman, unpubl. data for *D. tenebrosa*).

emergence, flies were aged for one week at 20.5°C and then spot numbers were counted. Spot numbers were analyzed as a function of rearing temperature and male-female pair type.

Correlation between Body Size and Spot Number

To determine whether there is a genetic correlation between body size and abdominal spot number, it is not appropriate to examine wild flies, as rearing temperature can affect both traits independently. Therefore, a sample of 20 males and 20 females of *D. falleni* was collected from Mendon Ponds Park in August 2003 and placed in mass culture in a population cage at 22°C. The F1 were aged for one week at 22°C and then scored for abdominal spot number and thorax length, which was measured at 25× using an ocular micrometer. An analysis of covariance was used to examine spot number as a function of fly sex and thorax length using JMP (SAS Institute 2001).

Fitness Assays

The flies used in fitness assays were obtained from six spotless strains and six wild-type strains. Female fecundity was measured by placing newly emerged spotless or wild-

type females individually with two males in cultures containing blended mushroom-agar-sucrose-yeast medium. Every two days the flies were transferred to fresh vials, and the eggs that had been laid were counted. A total of 46 wild-type and 44 spotless females was monitored in this way for six weeks, at which time all but four spotless and five wild-type females had died. Two measures of fecundity were computed: total lifetime fecundity, and mean number of eggs laid per day while the female was alive. Female longevity was monitored during this experiment by recording the day when a female died.

Development time was measured by setting up 12 vials each using as parents: (1) wild-type female × wild-type male, (2) spotless female × spotless male, (3) wild-type female × spotless male, and (4) spotless female × wild-type male. Each culture was initiated with 15 eggs that been collected from mushroom-agar medium. The larvae developed on a piece of *Agaricus bisporus* mushroom placed on top of moistened cheesecloth. More mushroom was added as needed to prevent food deprivation. Cultures were kept at 20.5°C.

Resistance to Parasite Infection

In natural populations, *D. falleni* is commonly infected with the parasitic nematode *Howardula aoronymphium* (Jaenike 1992, 2002). Inseminated female nematodes infect fly larvae by invading through the fly cuticle. Each successful infection results in the development of a mature motherworm that can be identified within adult flies. Thus, the number of motherworms within adult flies is a measure of infection rates by these nematodes. To assess whether wild-type and spotless flies differ in their susceptibility to *H. aoronymphium* infection, larvae of the two types were exposed simultaneously to these nematodes. The parents of the wild-type flies used in the experiment were obtained by pooling flies from seven wild-type strains, and those used as parents of spotless were obtained by pooling flies from seven spotless strains. All strains had been maintained in the laboratory for about one year prior to the experiment. Eggs were collected on mushroom-agar medium. Experimental infections were set up as follows: *D. falleni* adults infected with *H. aoronymphium* were ground in *Drosophila* Ringer's solution (Roberts 1986) and a quantity of slurry containing about 200 larval nematodes was pipetted onto a 0.1-g piece of *A. bisporus* mushroom. One day later, 15 eggs from wild-type flies and 15 from spotless flies were placed on the mushroom. A total of 56 replicate cultures was set up, each containing infective *H. aoronymphium* and eggs from both spotless and wild-type flies. The mushroom was then placed in a vial on top of moistened cheesecloth. The use of a small piece of mushroom presumably enhances the rate of contact between infective nematodes and fly larvae. As the larvae consumed the mushroom, additional mushroom was added to prevent food deprivation. Cultures were kept at 20.5°C.

All emergent adults were collected and aged for one week, at which time their abdominal phenotype was determined. The clearly bimodal distribution of spot numbers allowed classification of flies into spotless and wild-type categories, although many of the flies in the spotless category did have a few abdominal spots. The flies were then dissected to determine how many motherworms had infected them. For each culture vial, the mean number of motherworms per emergent fly was calculated separately for wild-type and spotless flies. From this, we calculated the relative susceptibility of spotless to wild-type flies within a culture as log([spotless mean/wild-type mean]). Positive values indicate that the spotless flies within a culture were infected with more motherworms, on average, than the wild-type flies, whereas negative values indicate the reverse. The distribution of these values across all 56 cultures was examined using a Wilcoxon signed-ranks test (as implemented in JMP; SAS Institute 2001), testing the null hypothesis that the mean of the distribution did not differ significantly from zero; that is, that spotless and wild-type flies were equally susceptible to parasitism.

Results

Wild-Caught Flies

The distribution of spot numbers in wild-caught flies is shown in Figure 2, and an ANOVA of the source of spot number variation is presented in Table 1. For males, the mean ± standard deviation of spot number for the four collections were 15.42 ± 1.93, 15.52 ± 1.85, 16.04 ± 1.40, and 16.11 ± 1.30, and for females these values were 18.30 ± 1.72, 18.34 ± 1.36, 18.35 ± 1.50, and 18.78 ± 1.16. For both sexes, there were several individuals with very few spots, including one completely spotless male. Several factors had a significant effect on spot number (Table 1). By far the greatest effect was sex, the mean ± standard error spot number of males being about 15% less than that in females (15.77 ± 0.06 versus 18.44 ± 0.05; Fig. 2). The second most important factor contributing to spot number variation was collection year, with flies collected in 1996 having fewer spots than those collected in 1997 (17.05 ± 0.06 vs. 17.49 ± 0.05). This was probably because the flies collected in 1996 had developed at warmer temperatures than those collected in 1997; in the three weeks prior to the July 1996 collection, when most of the collected flies probably developed, the mean temperature in Rochester was 20.4°C, whereas in the three weeks prior to the June 1997 collection the mean temperature was 18.0°C (National Climatic Data Center 2003). As we show below, spot number is inversely related to rearing temperature. There was a minor, and marginally significant, effect of site, with the flies from Mendon Ponds having slightly more spots than those from Cobbs Hill (17.35 ± 0.05 vs. 17.17 ± 0.06).

Selection for Reduced Spot Number

In comparison to the six control populations, all 13 selected populations exhibited substantial declines in mean spot number after 10 generations of selection (Fig. 3). The standard deviation of spot number within sexes in the control populations ranged from 0.96 to 2.61, whereas the standard deviation within sexes in the selected populations ranged from 1.7 to 3.56. The mean spot number in each of the selected lines was significantly less than in any of the controls ($P <$ 0.05; Tukey-Kramer HSD test, as implemented in JMP [SAS Institute 2001]; Fig. 3). In seven of the 13 selected popula-

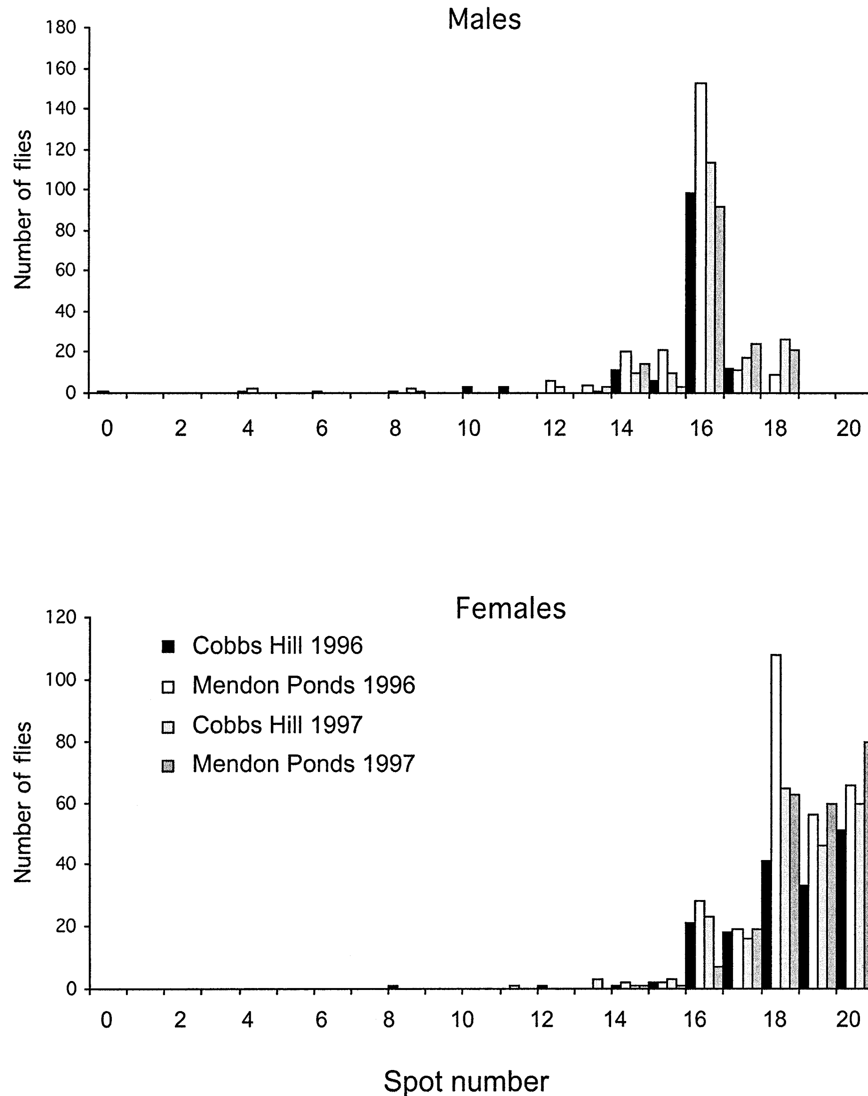

FIG. 2. Spot number distribution in wild flies, plotted separately for males and females.

tions, the mean spot number was less than half that in the control population with the fewest spots.

In nine of the selected populations, some completely spotless flies appeared, generally after two to five generations of selection. Furthermore, in 10 of the 13 selected populations, some flies with abnormal, poorly sclerotized patches of abdominal cuticle were produced, first appearing between generations 2 and 7. None of the six control populations produced any spotless flies or flies with abnormal abdominal cuticle. Even though each population was started with only 35 wild flies, there is clearly a great deal of heritable genetic variation for spot number within local populations of *D. falleni*.

TABLE 1. Sources of spot number variation among wild-caught *Drosophila falleni*.

Source	df	SS	*F*	*P*
Site	1	9.6654	4.1652	0.0414
Year	1	70.8814	30.5454	<0.0001
Sex	1	2702.1895	1164.472	<0.0001
Site × year	1	3.1281	1.3480	0.2458
Site × sex	1	2.0659	0.8903	0.3455
Year × sex	1	11.5877	4.9935	0.0256
Site × year × sex	1	4.2776	1.8434	0.1747
Error	1596	3703.5631	2.321	

In general, though not invariably, spot loss proceeded as follows. Initially, the abdominal spots became smaller and fainter. Next, the spots vanished on the more posterior abdominal segments, then on segments 2 and 4, and finally on segment 3. Within a segment the lateral spots were generally lost before the more medial spots (Fig. 4). This pattern is consistent with there being a single gradient of either substrate or enzyme whose concentration is greatest in the medial portion of segment 3 and that falls off laterally, anteriorly, and posteriorly. The spotless flies appear to be normal with respect to the level of clouding on the anterior and posterior crossveins and in the color of their thorax, which is yellowish-brown, suggesting that the effects of selection on pigmentation are specific to the abdomen.

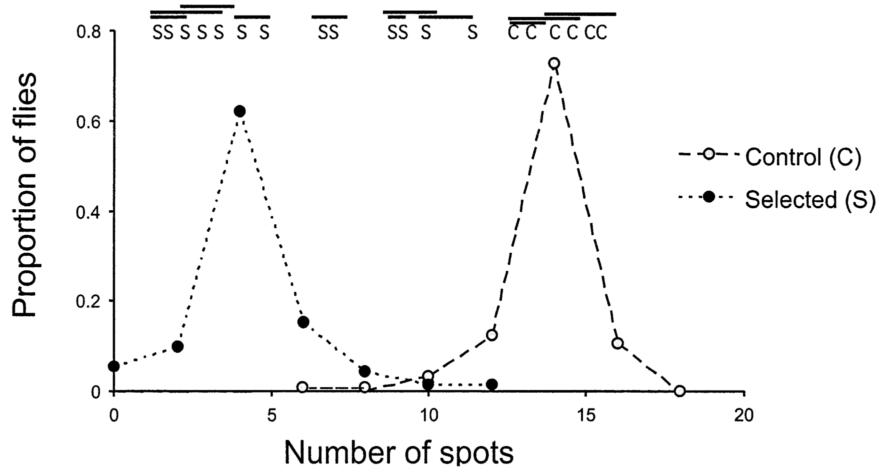

FIG. 3. Results of selection experiment. Representative spot number distributions of males from control and selected populations are shown. The S and C values above these distributions indicate the mean spot numbers of flies in the different populations after 10 generations of selection. Horizontal lines cover population means that are not significantly different ($P > 0.05$).

After 15 generations of additional culture with no selection on spot number, spot number was surveyed in the surviving populations. Remarkably, the mean spot number at generation 25 was almost exactly the same as in generation 10 ($r = 0.94$ for selected populations only, $r = 0.98$ including selected and control populations; Fig. 5). A fitted regression for the selected populations only yields a slope of 0.95 ± 0.11, and a Y-intercept of 0.63 ± 0.95, values not significantly different from a slope = 1 and Y-intercept = 0. Thus, there was no evident tendency of the selected lines to revert to the wild-type spot number when selection was relaxed.

Effect of Rearing Temperature

Larval rearing temperature had a highly significant effect on spot number in week-old adult flies (Table 2). Flies reared at lower temperatures had significantly more spots than those reared at higher temperatures (Fig. 6). As expected, there was a significant effect of fly pair, as some parents had wild-type spot numbers, others were spotless, and others were intermediate. Spot numbers in all three categories of flies showed qualitatively similar responses to rearing temperature.

Correlation between Body Size and Spot Number

Among the lab-reared offspring of wild-caught flies, spot number ranged from 10 to 20 among females (mean = 16.30; SD = 2.03; $n = 79$) and from zero to 16 among males (mean = 12.07; SD = 3.06; $n = 91$). Spot number differed significantly between male and female offspring of wild-caught flies ($F = 32.7$; $P < 0.0001$). However, there was no correlation between spot number and thorax length within sexes ($F = 1.39$; $P = 0.66$). These patterns indicate that abdominal spot number is genetically independent of body size.

Fitness Assays

Female fecundity and longevity.—Wild-type and spotless females laid very similar numbers of eggs, both on a daily basis (spotless $\bar{x} = 17.72 \pm 1.28$ per day; wild-type $\bar{x} = 18.73 \pm 0.87$ per day) and over their lifetimes (spotless $\bar{x} = 431 \pm 39$; wild-type $\bar{x} = 435 \pm 35$). Female longevity did not differ between the two types of females (spotless $\bar{x} = 25.9 \pm 1.5$ days; wild-type $\bar{x} = 24.9 \pm 1.5$ days). Nested ANOVAs showed that strain (nested with phenotype cate-

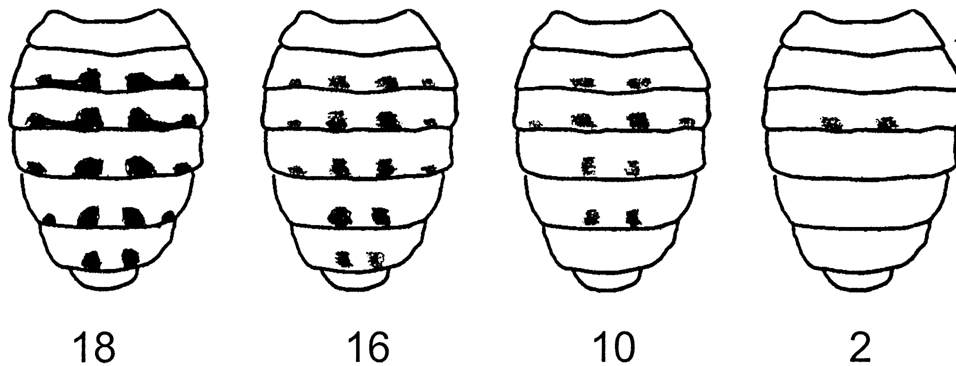

FIG. 4. Variation in spotting patterns among *Drosophila falleni* males, showing from left to right flies with 18, 16 (the modal number of spots among wild-caught males), 10, and two spots. Note that as spot number decreases, spots become smaller and fainter and are lost from the posterior abdominal segments and the lateral margins of more anterior segments.

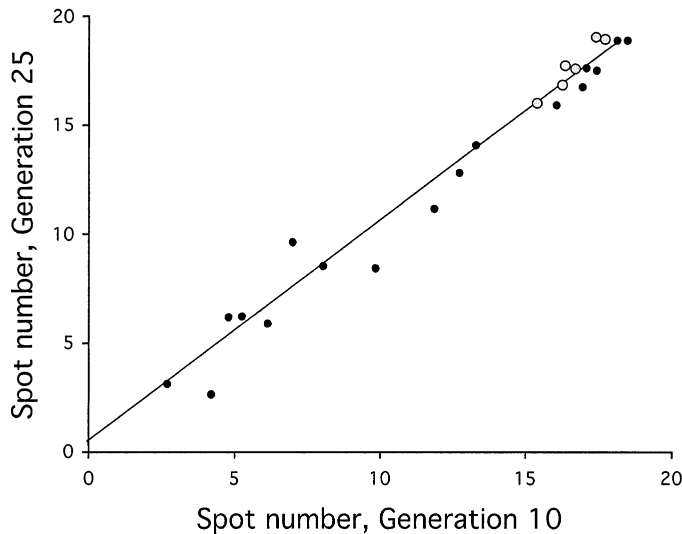

FIG. 5. Correlation between mean spot numbers after 10 generations of selection and at generation 25, after 15 subsequent generations in which there was no selection. The solid line is a least-squares linear regression, showing that there was very little change from generation 10 to generation 25. Selected populations shown in black, control populations in gray.

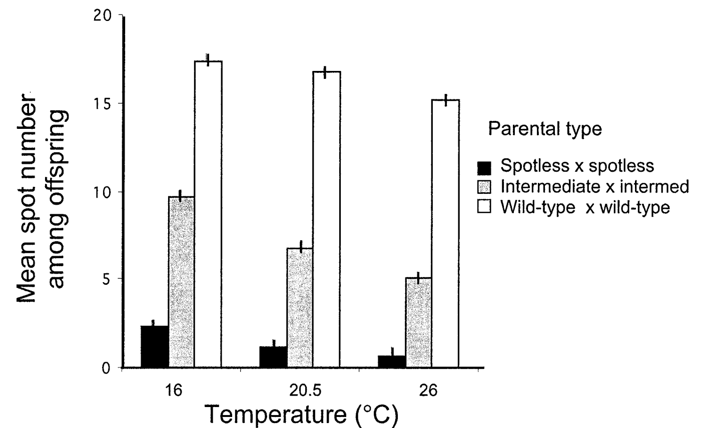

FIG. 6. Effect of rearing temperature on spot number of flies, using wild-type, intermediate, and spotless flies as parents.

gory) had a significant effect on life span and lifetime fecundity, but not on daily fecundity. For none of the three fitness measures, however, was there a significant difference between spotless and wild-type flies (daily fecundity: F = 0.17, P = 0.68; lifetime fecundity: F = 0.0, P = 0.99; longevity: F = 0.22, P = 0.64).

Development time.—The mean egg-to-adult development time of males was slightly but significantly longer than that of females (16.13 ± 0.09 vs. 15.89 ± 0.10; $P < 0.001$; Table 3). Although mean development times differed among replicate cultures within cross types, there were no significant differences observed as a function of the type of parents used: neither female parent type, male parent type, nor a female X male interaction was evident (Table 3). For instance, the mean development time of offspring of wild-type × wild-type crosses was 15.90 ± 0.05, whereas that of spotless × spotless was 15.97 ± 0.14.

Resistance to Parasite Infection

The distribution across cultures of the ratio (spotless mean/wild-type mean), log-transformed, is shown in Fig. 7. Positive values indicate that the mean number of motherworms per fly was greater for spotless than wild-type flies within a culture. A Wilcoxon signed-ranks test reveals that the mean of log([spotless mean/wild-type mean]) distribution is significantly different from zero (t = 3.57; $P > |t|$ = 0.0008). On average, spotless flies were infected with 1.78 ± 0.16 as many motherworms as were wild-type flies within cultures.

DISCUSSION

Our surveys reveal that natural populations of *D. falleni* exhibit considerable variation in abdominal spot number. Some of the variation among individuals in nature is undoubtedly due to their having experienced different environmental conditions during development. For instance, we found that spot number was inversely correlated with rearing temperature in the laboratory, and temperature is the most likely cause of the difference in mean spot number between the July 1996 and June 1997 collections. The difference in temperature in the three-week periods prior to the 1996 and 1997 collections (20.4°C vs. 18.0°C) is likely to have been sufficient to cause the small but significant difference in mean spot number between the two years. Studies of other North American species of mycophagous *Drosophila*, including *D. putrida* (Sabath et al. 1973), *D. neotestacea* (K. A. Dyer, pers. comm.), and *D. innubila* (*J. jaenike*, unpubl. data), show that the darker pigmentation of individuals that developed at low temperature is a consistent feature of these flies.

Our studies also showed that there is a great deal of genetic variation for abdominal pigmentation in *D. falleni*, as evidenced by the consistent, rapid, and substantial response to selection for reduced spot number. In most cases, selection led to populations many standard deviations from the original

TABLE 2. Effect of rearing temperature (16°C, 20.5°C, or 26°C) on abdominal spot number in *Drosophila falleni*.

Source	df	SS	F	P
Pair	59	2887.0654	10.4969	<0.0001
Temperature	1	1334.9611	286.3683	<0.0001
Sex	1	1.0036	0.2153	0.6427
Pair × temperature	59	962.2623	3.4986	<0.0001
Pair × sex	59	480.2128	1.7460	0.0005
Temperature × sex	1	75.3815	16.1704	<0.0001
Pair × temperature × sex	59	564.4690	2.0523	<0.0001
Error	1258	5864.409		

TABLE 3. Effect of parent type (spotless or wild-type) on offspring development time.

Source	df	SS	F	P
Female parent	1	0.99	1.30	0.26
Male parent	1	0.16	0.20	0.65
Female × male	1	0.01	0.02	0.89
Offspring sex	1	42.72	55.91	<0.0001
Vial (female, male)[1]	36	163.4	5.94	<0.0001
Error	1036	967.0		

[1] Replicate vials within specific combinations of male and female parental types.

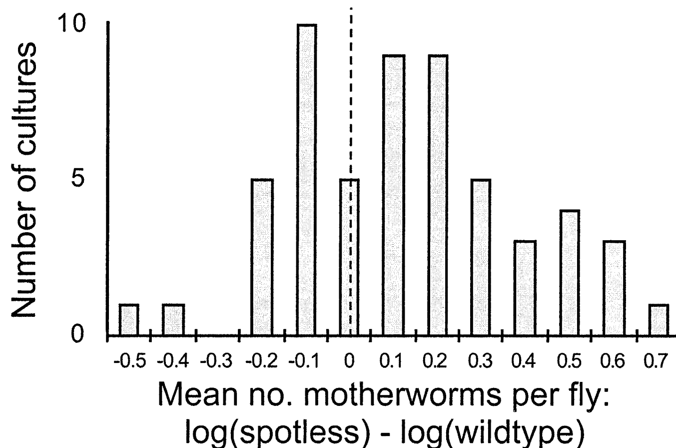

FIG. 7. Within-culture intensity of parasitism of spotless flies relative to wild-type flies. For each culture the mean number of motherworms per fly was determined for the two types of flies. To show relative differences in parasitism, the means were $\log_{10}$-transformed. The difference in the transformed values, [$\log_{10}$(spotless) − $\log_{10}$(wild-type)], equals the $\log_{10}$ of the ratio of mean number of motherworms in the two types of flies. Values greater than zero indicate that the mean number of motherworms within a culture was greater in spotless than in wild-type flies.

mean, indicating that populations of *D. falleni* have the potential to evolve a level of phenotypic divergence that characterizes different species of the *quinaria* group. The fact that the replicate selected lines reached significantly different final spot numbers, and the complete lack of response to relaxed selection suggests that different combinations of alleles affecting spot number may have became fixed in the different lines.

In 10 of the 13 selected populations, some individuals manifested abnormal patches of abdominal cuticle lacking both pigmentation and sclerotization. No such individuals were found in any of the six control populations. Thus, the production of abnormal cuticle is a correlated response to selection for reduced spot number. The consistent appearance of this phenotype in the selected lines suggests that pleiotropy, rather than linkage disequilibrium, underlies the correlated response to selection. Among the candidate genes for such pleiotropic effects are those that encode enzymes involved in melanization and sclerotization. Such enzymes include tyrosine hydroxylase, Dopa decarboxylase, and phenol oxidase (Wright 1987). A second possibility is the rDNA locus, which is associated with cuticular abnormalities in other species of *Drosophila* (Templeton et al. 1985; Malik and Eickbush 1999), and which can be highly polymorphic in natural populations (Lyckegaard and Clark 1991; Perez-Gonzalez and Eickbush 2001).

Although the external appearance of our selected, spotless flies is very different from typical wild-type flies, we detected no statistically significant effects of spotlessness on basic demographic variables in our laboratory assays: spotless and wild-type flies were very similar with respect to egg-to-adult development time, adult survival, and female fecundity. Under different environmental conditions, it is possible that spotless and wild-type flies would differ with respect to these components of fitness. Spotless flies were, however, significantly more susceptible to infection by the nematode *Howardula aoronymphium*. The greater rate of parasitism of spotless flies is probably not due to their having a longer period of exposure to parasites, as egg-to-adult development times were very similar for spotless and wild-type flies. These nematodes commonly infect *D. falleni* in nature, and a 14-year field study has shown that the average prevalence of infection of this species is 11% around Rochester, New York (Jaenike 2002). Infection with *H. aoronymphium* causes about a 50% reduction in the fertility of *D. falleni* females (Jaenike 1992). Thus, these nematodes are likely to represent an important selective factor in natural populations of *D. falleni*.

To reduce the cost of nematode parasitism, flies could either suppress the deleterious effects of infection, for instance by encapsulating an invading parasite, or resist becoming infected in the first place. *Drosophila* larvae become infected with *H. aoronymphium* when the nematodes invade through the cuticle (Welch 1959). One possible connection between spot number and resistance to nematode infection is that the alleles causing spotlessness and cuticular abnormalities in adult flies might also make the larval cuticle a less effective barrier to nematode infection. Alternatively, a correlation between abdominal pigmentation and parasite resistance could be related to levels of melanin production, as melanization is involved in the parasite encapsulation reactions of some insects (Lavine and Strand 2002). Although we found no encapsulated nematodes within the adult flies dissected, it is possible that fly larvae mounted an immune response against the nematodes immediately after becoming infected. Because the nematodes are extremely small at this point, one might overlook melanized or encapsulated nematodes, even if they persisted into the adult flies.

Recent models of the coevolution of host resistance and parasite virulence (Sasaki and Godfray 1999; Roy and Kirchner 2000; Jokela et al. 2000) predict the existence of a genetic polymorphism for resistant and susceptible types within a host population, rather than quantitative variation for resistance. Our results on *D. falleni* suggest that quantitative variation in resistance to nematode infection can arise as a pleiotropic consequence of quantitative variation for other traits, in this case abdominal spot number. Whatever the underlying genetic basis for spot number variation, selection on abdominal pigmentation in *D. falleni* may bring about quantitative variation in resistance to *H. aoronymphium* infection and vice versa.

The abundant genetic variation for abdominal spot number

within natural populations of *D. falleni* and the apparent lack of effect on fly development, survival, and fecundity suggests that this species could undergo rapid evolutionary change in external appearance, given the appropriate selective conditions. Although the *quinaria* group is evolutionarily young (Perlman et al. 2002), it has already undergone substantial differentiation among species in patterns of wing and abdominal pigmentation (see Fig. 1). If *D. falleni* is typical of the group, then levels of genetic variation are unlikely to limit the rate of further phenotypic diversification in the *quinaria* group.

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