The Genetic Basis of *Drosophila sechellia*'s Resistance to a Host Plant Toxin

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ABSTRACT

Unlike its close relatives, *Drosophila sechellia* is resistant to the toxic effects of the fruit of its host plant, *Morinda citrifolia*. Using 15 genetic markers, I analyze the genetic basis of *D. sechellia*'s resistance to this fruit's primary toxin, octanoic acid. *D. sechellia*'s resistance is dominant in F_1 hybrids between it and its sister species *D. simulans*. All chromosomes, except the *Y* and the dot fourth, carry genes affecting resistance. The third chromosome has the greatest effect and carries at least two factors. The *X* chromosome has an intermediate effect and harbors at least two genes, whereas the second chromosome carries at least one gene of weak effect. Thus, at least five loci are involved in this adaptation. However, I also identified large chromosome regions having no effect on resistance, suggesting that *D. sechellia*'s resistance is neither very simple nor highly polygenic. Instead, resistance appears to have an oligogenic basis. *D. sechellia*'s resistance to its host may contribute to ecological isolation between it and *D. simulans*.

ALTHOUGH it is an essential aspect of biology, we know little about the genetic basis of adaptation (Orr and Coyne 1992). An understanding of the genetics of adaptation requires that we answer at least three questions: (1) How many genes are typically involved in the evolution of a new adaptation? (2) What is the distribution of phenotypic effects among these genes? (3) What are the roles of dominance and epistasis?

We have fairly good answers to these questions for adaptations to economically important agricultural pesticides (Bishop 1981; Forgash 1984; Roush and McKenzie 1987; Roush 1993; Carriere and Roff 1995; McKenzie and Batterham 1995). Pesticide resistance typically involves one or a few genes of large phenotypic effect (Roush and McKenzie 1987; Raymond *et al.* 1991; Orr and Coyne 1992; Roush 1993; ffrench-Constant 1994; Carriere and Roff 1995; McKenzie and Batterham 1995; Pasteur and Raymond 1996). When more than one gene is involved, epistatic interactions are common (Plapp 1984; Houpt *et al.* 1988; Roush 1993; McKenzie and Batterham 1995). Finally, resistance factors are often dominant or codominant to their susceptible allelomorphs (Ottea and Plapp 1984; Houpt *et al.* 1988; Roush and McKenzie 1987; Roush 1993; ffrench-Constant 1994).

Similarly, we know a good deal about the genetics of industrial melanism, heavy metal tolerance, disease and herbicide resistance in plants, and host plant preferences in pest species (Kettlewell 1973; Grant *et al.* 1996; MacNair 1993; Jasieniuk *et al.* 1996; Martinez and Levinton 1996; Schat *et al.* 1996; Ashfield *et al.* 1995; Staskawicz *et al.* 1995; Gould 1988; Sheck and Gould 1996). Again, these adaptations typically involve one or a few dominant genes of large phenotypic effect.

Unfortunately, all of the above examples reflect adaptations to human disturbance. Given that selection pressures experienced during such disturbances may differ profoundly from those characterizing selection in the wild, we cannot necessarily generalize from the genetic basis of such adaptations to the genetic basis of more "natural" adaptations.

Theoretical population genetics has similarly struggled to characterize the genetics of natural adaptations. Fisher's (1930) classic geometric model of adaptation suggested that adaptations are built from many genes of small phenotypic effect each. Kimura (1983), however, later argued that—when one takes into account the probability of fixation of favorable mutations—adaptations should be built from fewer genes, each having a more intermediate phenotypic effect. Since then, others have constructed quantitative models of adaptation (Lande 1983; MacNair 1991; Bürger 1991, 1993). But as Orr and Coyne (1992) point out, none of these models is entirely satisfactory as each involves unrealistic assumptions or ignores potentially important population genetic forces. Given this lack of theoretical consensus, only empirical analysis will clarify the genetic basis of natural adaptations.

Although a few studies have begun to shed light on natural adaptations (Bradshaw *et al.* 1995; Bradshaw and Stettler 1995; Chakir *et al.* 1996; Liu *et al.* 1996; Mitchell-Olds 1995, 1996; Shoemaker and Ross 1996; Orr and Irving 1997; True *et al.* 1997), we still suffer from an astonishing shortage of rigorous genetic analyses of adaptation. This shortage reflects two problems. First, many interesting experimental systems with dramatic or obvious adaptations have historically lacked the tools required for genetic analysis. Second, in sys-

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tems in which such tools were available, the adaptive significance of many traits remained unclear (reviewed in Orr and Coyne 1992). Although molecular marker-based quantitative trait locus (QTL) analysis has provided the needed genetic tools for many species, most studies to date have been limited to either agricultural or medical systems (for exceptions, see discussion).

Here I study the genetic basis of an unambiguous natural adaptation in *Drosophila sechellia*. First described in 1981, *D. sechellia* is endemic to the Seychelles archipelago in the Indian Ocean (Tsacas and Bachli 1981). It is morphologically almost identical to its cosmopolitan sister species *D. simulans*, and to the island endemic *D. mauritiana* (Tsacas and Bachli 1981). When crossed to either of these species, *D. sechellia* produces fertile hybrid females and sterile hybrid males (Lachaise *et al.* 1986). When crossed to its more distant relative, *D. melanogaster*, *D. sechellia* produces only sterile or inviable progeny (Lachaise *et al.* 1986).

On its native islands, *D. sechellia* specializes on the fruit of *Morinda citrifolia* (Tsacas and Bachli 1981; Lachaise 1983; Lachaise and Tsacas 1983; Louis and David 1986). Lachaise *et al.* (1988) suggest that *D. sechellia* may have specialized on Morinda to escape competition from other species of Drosophila. Because its sister species are all generalists, *D. sechellia* is believed to have evolved its host specialization after its ancestor invaded the Seychelles (Lachaise *et al.* 1988).

R'Kha *et al.* (1991) performed a preliminary genetic analysis of *D. sechellia* adult resistance to Morinda fruit. Crossing *D. sechellia* to *D. simulans*, they showed that resistance was dominant to susceptibility. Using a biometric approach, they estimated the number of effective factors to be three to five (R'Kha *et al.* 1991). Because R'Kha *et al.*'s study did not employ any genetic markers, resistance factors could not be mapped.

Subsequently, Legal *et al.* (1992, 1994) and Farine *et al.* (1996) identified octanoic acid as the compound causing Morinda's lethal effect on adult flies. These studies showed that octanoic acid represents 58% of identifiable volatile compounds in Morinda (Farine *et al.* 1996). Legal *et al.* further showed that ripe fruit, which is the most toxic to Drosophila, contained much more octanoic acid than unripe or rotten fruits. Finally, they demonstrated that commercially available octanoic acid has toxic effects similar to those of Morinda fruit. Hexanoic acid, which is present in the fruit in lower quantities, was shown to also affect the flies, but not nearly as severely as octanoic acid (Farine *et al.* 1996).

Amlou *et al.* (1997) repeated the analysis of R'Kha *et al.* (1991) using octanoic acid and hexanoic acid. Resistance was found to be dominant. Again, resistance factors were not mapped.

Because *D. sechellia* and its sister species provide many genetic tools and because *D. sechellia*'s resistance to Morinda is obviously adaptive, the *D. sechellia*/Morinda case provides an ideal system for genetic analysis of adaptation. This article reports the first step in a two-step analysis of the genetics of *D. sechellia*'s resistance to Morinda's toxin. Through a series of interspecific backcrosses employing 15 visible markers, I mapped the chromosome regions harboring Morinda/resistance factors. These data provide information on the complexity of *D. sechellia*'s resistance and on the possible role of major factors in this adaptation. Also, these results identify chromosome regions that must be further dissected in future molecular marked-based analyses of resistance, as well as those that can be ignored. This is the first in a series of genetic analyses of traits underlying *D. sechellia*'s adaptation to its host plant.

MATERIALS AND METHODS

Crosses: Like Coyne (1996), I localized resistance factors by crossing a multiply marked (recessive) stock of *D. simulans* females to *D. sechellia* males. The resulting F_1 females were then backcrossed to males from the *D. simulans* marker strain. These backcross progeny carry, on average, $\frac{3}{4}$ of their genes from *D. simulans* and $\frac{1}{4}$ from *D. sechellia*. Because recombination acts in F_1 females, markers identify the species origin of chromosome regions, not of entire chromosomes. Expression of recessive markers indicates chromosome regions that are homozygous for material from *D. simulans*; wild-type phenotypes indicate chromosome regions that are heterozygous for *D. sechellia* and *D. simulans* material. All map distances are literature values except for those on chromosome *3*, which was recently remapped (Jones and Orr 1998). Details of each cross are provided in the results section.

Stocks: Stocks used are described in Table 1. All flies were reared at 24° on agar-yeast-cornmeal medium.

Resistance assay: Resistance to octanoic acid was scored as resistance to knockdown, which is a prelude to fly death (data not shown). To test knockdown, 1.5 μl of octanoic acid (Sigma Chemical Co., St. Louis) was placed on the lid of a 15 × 60 mm polystyrene petri dish. Five-day-old flies were very lightly gassed with CO_2 and placed in the dish. Knockdown was scored at regular time intervals. Percent knockdown at 60 min is reported here. All tests were conducted at 22° (±0.5). Both sexes were treated identically.

Following ffrench-Constant *et al.* (1992), I used percent survival after a period of time, rather than LT50, as the measure of resistance. Because *D. sechellia* is very resistant to octanoic acid (relative to *D. simulans*), LT50 measurements are impractical and potentially misleading. Percent survival, on the other hand, provides a simple metric that readily distinguishes the resistance of the parental species and backcross progeny.

Statistics: Most backcross data were analyzed using the FUNCAT procedure in SAS (SAS Institute, Inc., Cary, NC). This procedure finds the effect of substituting a chromosome region by comparing all genotypes that differ in that region. The result is reported as a χ^2 statistic. For all other pairwise comparisons, I used contingency table analysis, which is reported as a χ^2 statistic (Sokal and Rohlf 1995).

RESULTS

Within-species resistance: Four wild-type isofemale lines of *D. sechellia* were tested for resistance to octanoic acid. All were highly resistant with no significant differ-

TABLE 1

Strains used

Species	Stock	Comment
D. mauritiana	Synthetic	A mixture of six isofemale lines collected by O. Kitagawa on Mauritius in 1981 and pooled in 1983 from J. Coyne
	y w	See Linsey and Zimm (1992)
D. melanogaster	*y w*	"Robertson." Collected from Seychelles in 1981 by Tsacas and Bachli. A wild-type isofemale line
D. sechellia	Line 1	Collected in the Seychelles by J. R. David in 1985
	Lines 4, 24, and 81	From J. Coyne
	cn pr	From J. Coyne
	zn v f	Wild-type line collected in Islamorada, FL
D. simulans	Islamorada	Wild-type line from A. H. Sturtevant's Caltech stock collection
	Solway-Hochman	From J. Coyne. See Coyne and Berry (1994)
	ey	From J. Coyne
	f; nt pm; st e	From J. Coyne
	nt b py sd pm	From J. Coyne
	y w m f	Constructed from stocks provided by J. Coyne
	jv st e pe	Constructed from stocks provided by J. Coyne
	jv st e osp pe	Constructed from stocks provided by J. Coyne
	st e osp	From Species Stock Center
	C(1) RM, y w/inc^{1}	From Species Stock Center
	C(1)RM, y w/C(1;Y) AB/0	

ences in resistance (Table 2). Two mutant marker stocks of *D. sechellia* were also tested for resistance. Again, no differences were found (Table 2). Resistance to octanoic acid, therefore, appears to be general to *D. sechellia*.

Interspecific comparisons: R'Kha *et al.* (1991) showed that *D. sechellia* is much more resistant to Morinda than is *D. melanogaster*, *D. mauritiana*, or *D. simulans*. To verify that this species difference extends to resistance to pure octanoic acid, I tested several isofemale lines of *D. sechellia* and its three sisters species. Figures 1 and 2 present survival curves for these lines. The difference between *D. sechellia* and the other species is large and highly significant: All non-*sechellia* strains were much more susceptible to octanoic acid than was *D. sechellia* (comparison of "worst" *D. sechellia* line to "best" non-*sechellia* line: males $\chi^2 = 81.33$, $P < 0.0001$; females: $\chi^2 = 86.55$, $P < 0.0001$). The degree of susceptibility did, however, vary somewhat among the different strains of the susceptible species (Figures 1 and 2).

TABLE 2

Within-species resistance to octanoic acid

D. sechellia line tested	Total tested	Total knockdown	% survival
Males			
Line 1	109	1	99
Line 4	102	0	100
Line 24	112	0	100
Line 81	107	4	96
zn v f	51	0	100
cn pr	93	1	99
Females			
Line 1	106	1	99
Line 4	109	2	98
Line 24	112	0	100
Line 81	114	4	96
zn v f	43	0	100
cn pr	82	4	95

It is important to note that mutant-marked and wild-type strains showed similar levels of resistance within each species (see Table 2 and Figures 1 and 2). Thus a fly's resistance depends on its species identity, not on the presence or absence of markers.

Resistance in F_1 hybrids: R'Kha *et al.* (1991), showed that resistance to Morinda fruit is dominant. Amlou *et al.* (1997) showed that resistance to pure octanoic acid is also dominant. I confirmed this result using my assay and stocks: Pure *D. sechellia* females and F_1 hybrid females [from the cross of *D. simulans f*^{2} (*1*-56.0); *nt* (*2*-0) *pm* (*2*-103); *st* (*3*-46.3) *e* (*3*-59.4) females to *D. sechellia* line 1 males] do not differ in resistance (Figure 3; $\chi^2 = 2.484$, $P = 0.115$). F_1 hybrid males, however, are less resistant than *D. sechellia* males ($\chi^2 = 24.422$, $P < 0.0001$). Because hybrid males are hemizygous for the susceptible *D. simulans X* chromosome, this result suggests that the *X* carries genes affecting resistance. These results are qualitatively similar to those of Amlou *et al.* (1997).

To further test the role of the sex chromosomes in resistance, I performed several crosses involving a compound-*X* from *D. simulans*. First, I used the parental stocks from the above analysis to produce F_1 hybrid males. Second, I crossed *D. simulans C(1)RM, y w* females to *D. sechellia* line 1 males and collected F_1 progeny. These F_1 males have an unrecombined *X* from *D. sechel-*

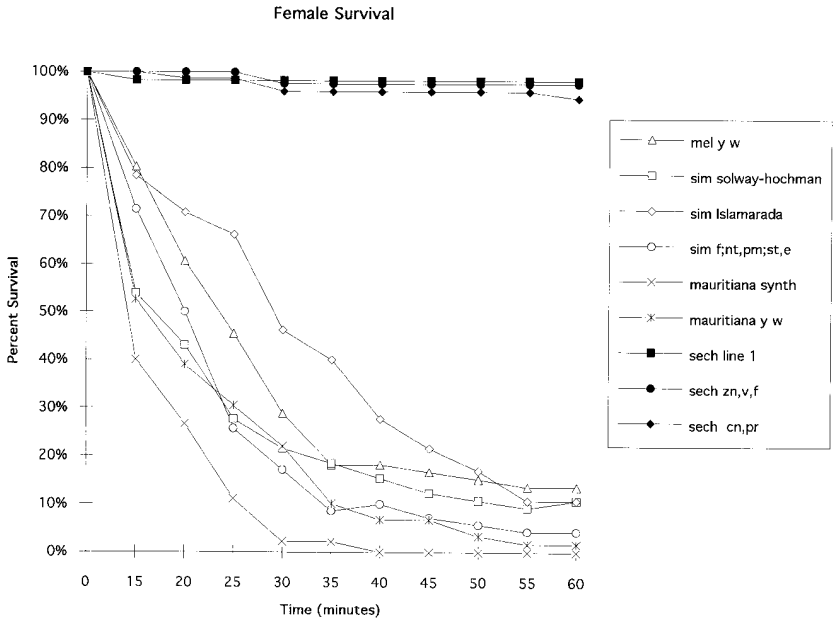

Figure 1.—Species comparison of male survival to octanoic acid.

lia, and a *Y* from *D. simulans.* To test for possible effects of the *D. simulans Y* chromosome, I repeated the above cross using *C(1)RM, y w/C(1;Y) AB/0.* This cross produces males with an unrecombined *D. sechellia X* but no *Y.*

Testing these three F_1 genotypes simultaneously (along with pure species controls), I found no significant differences among them (data not shown). The lack of effect of the unrecombined *D. sechellia X* chromosome suggests that, in males, the *X* has a less pronounced effect than that of the other chromosomes combined, though interpretation of these results is somewhat compromised by the strong resistance shown by all genotypes.

Not surprisingly, the *Y* chromosome has no effect on resistance. Because all of the hybrids in these crosses carry *D. simulans*'s cytoplasm, their resistance must be due to nuclear genes, not to any cytoplasmic factor.

"Whole" chromosome substitutions: By backcrossing F_1 hybrid females to *D. simulans*, I tested the effect of moving *D. sechellia* chromosomes into a mostly *D. simulans* background. This cross used *D. simulans* f^2; *nt pm* ; *st e* and *D. sechellia* line 1 (*pm* is a recessive allele of the *Punch* locus; Coyne 1983, 1984). With recombination, this backcross produces 32 distinguishable genotypes. Following Coyne (1983, 1984), I examined only those eight genotypes that roughly correspond to whole chromosome substitutions. That is, both chromosome arms carry *D. simulans* markers or both chromosome arms carry *D. sechellia* markers.

Figures 4 and 5 show that chromosomes *X*, *2*, and *3*

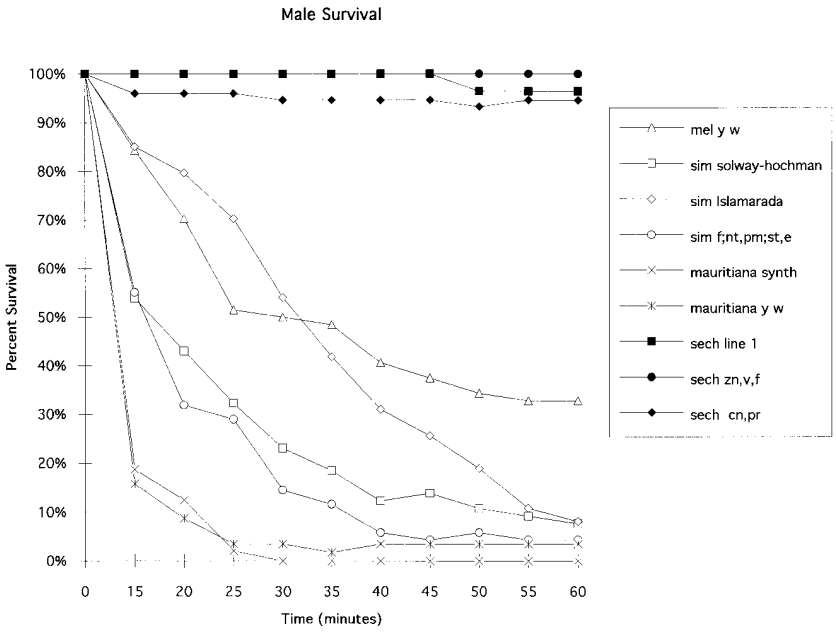

Figure 2.—Species comparison of female survival to octanoic acid.

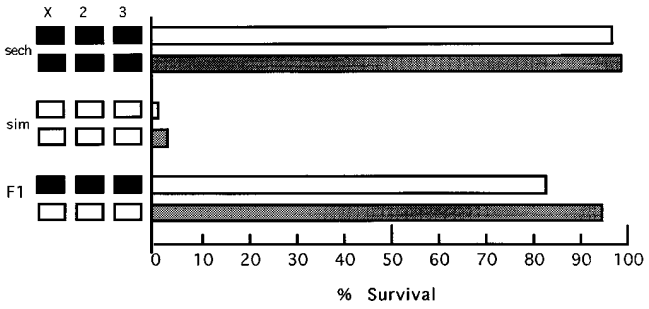

Figure 3.—Resistance to octanoic acid is dominant in F_1 hybrids. Solid rectangles represent *D. sechellia* chromosomes; open rectangles represent *D. simulans f; nt pm; st e* chromosomes. For *D. sechellia*, $N = 222$ males and $N = 212$ females were tested. For *D. simulans*, $N = 208$ males and $N = 233$ females were tested. For F_1 hybrids, $N = 213$ males and $N = 206$ females were tested. Open bars, male; shaded bars, female.

affect resistance in both males and females. FUNCAT analysis reveals that all of these effects are significant in both sexes (Table 3). Chromosome *3* has the greatest effect on resistance. The main effect of this chromosome explains 56% of the difference between the most *D. sechellia*-like genotype and the most *D. simulans*-like genotype in males (44% in females). The *X* chromosome has the next largest effect (17% in males, 28% in females), whereas chromosome *2* has the least effect (15% in males, 19% in females). Chromosome *4* was tested later (see below). Females of a given genotype tend to survive better than males, probably reflecting larger female size. Also, hybrid males may be less healthy when their *X* chromosome derives from one species and most of their autosomes from another. After correcting for size, these male flies have significantly lower resistance than their female equivalents (analysis not shown). The other genotypes are not different. Nevertheless, the genetic basis of resistance appears qualitatively similar in both sexes.

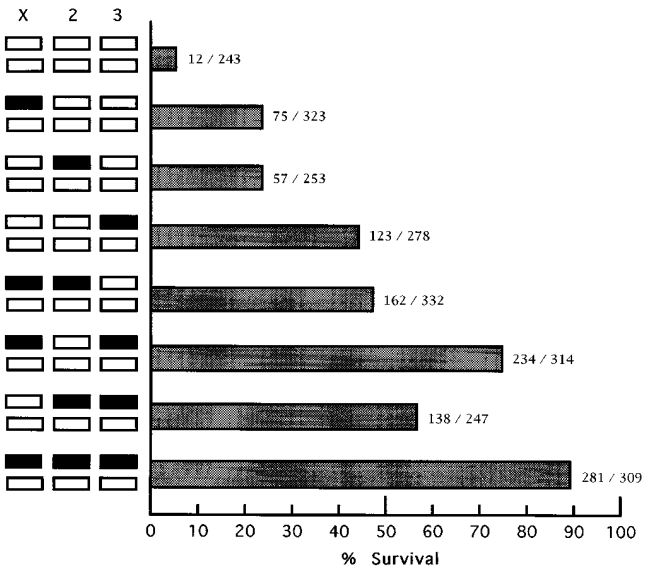

Figure 4.—Survival of male backcross progeny with "whole" chromosome substitutions (see text for explanation). Solid bars represent *D. sechellia* chromosomes; open bars are *D. simulans f; nt pm; st e* chromosomes.

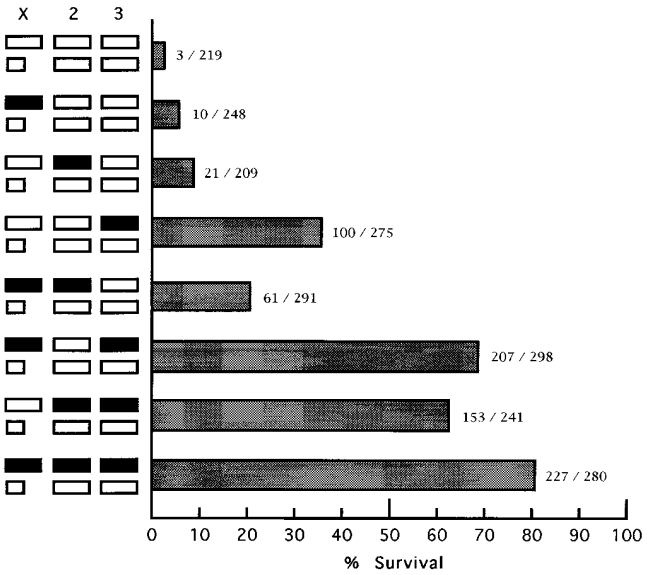

Figure 5.—Survival of female backcross progeny with "whole" chromosome substitutions (see text for explanation). Solid bars represent *D. sechellia* chromosomes; open bars represent *D. simulans f; nt pm; st e* chromosomes.

In both males and females, the *X* shows no significant interaction with any other chromosome (Table 3). Chromosome *2*, on the other hand, interacts with chromosome *3* in both sexes. Females, unlike males, show an additional significant epistatic interaction among all

TABLE 3

All chromosomes affect resistance

Chromosome	% effect or direction	Probability
Males		
X	17	<0.0001
2	15	<0.0001
3	56	<0.0001
*X*2*	None	0.3465
*X*3*	None	0.6849
*2*3*	Positive	0.0045
*X*2*3*	None	0.7567
Females		
X	28	<0.0001
2	19	<0.0001
3	44	<0.0001
*X*2*	None	0.6884
*X*3*	None	0.3706
*2*3*	Positive	0.0140
*X*2*3*	Positive	0.0049

Results of whole chromosome substitutions; that is, these analyses only use data in which all markers on a particular chromosome are either from *D. sechellia* or *D. simulans*. $N = 2061$ males; $N = 2299$ females.

three chromosomes. This sex difference may reflect the hemizygous *X* in males.

A few problems potentially complicate genetic analyses like those described above. First, one may confound susceptibility with hybrid inviability. That is, some genotypes may *appear* susceptible merely because they suffer from hybrid inviability caused by genic incompatibilities. Under this hypothesis, flies carrying all of their chromosomes from *D. simulans* would be the most fit and hence the least susceptible. In contrast, flies with a mixture of *D. simulans* and *D. sechellia* chromosomes would be less fit and more susceptible. My data, however, reveal the opposite pattern. Figures 4 and 5 show that flies having a *D. simulans*-like genotype are the *most susceptible*. Put differently, moving "foreign" *D. sechellia* chromosomes into an otherwise *D. simulans* background always *improves* viability (analysis not shown). Although hybrid inviability may have some quantitative effect on knockdown, the data strongly suggest that the large qualitative effects seen in Figures 4 and 5 reflect resistance genes.

Second, genetic markers might affect resistance. However, Coyne (1984) showed that the markers used in this analysis do not affect backcross hybrid size, eliminating the possibility that marked flies are more susceptible simply because they are smaller. More important, as noted above, comparisons of marked and unmarked lines in both species show that the presence *vs.* absence of markers has no effect on resistance to octanoic acid (Table 2).

Third, because hybrids produced in the above backcrosses are at most heterozygous for *D. sechellia* chromosomes, I cannot detect recessive *D. sechellia* resistance factors. Although resistance is dominant in F_1 hybrids, showing that recessive alleles are not required for *D. sechellia*-like levels of resistance, this result does not prove that recessive factors do not exist. Therefore, my estimates of gene number are minimum estimates. However, it is clear from Table 3 that I can explain at least 90% of the phenotype by dominant factors.

Finally, because F_1 females were used in these analyses, recombination between markers and resistance loci increases the chances that I will fail to detect factors that are loosely linked to markers. To remedy this problem and to further refine my mapping, I dissected each major chromosome using multiply marked chromosomes.

***X* chromosome:** To find regions affecting resistance on the *X* chromosome, I moved regions from the *D. sechellia X* into a mostly *D. simulans* background. To do this, I crossed *D. simulans y* (*1*-0.0) *w* (*1*-4.1) *m* (*1*-35.4) f^2 (*1*-56.0) females to *D. sechellia* line 1 males, backcrossed the F_1 females to the *D. simulans* parental strain, and tested the resistance of the resulting progeny. Of course, male flies were hemizygous for the *X*, whereas females were heterozygous or homozygous for different regions of the *X*. The remainder of the genome was, on average, ¾ *D. simulans*.

TABLE 4

Recombination analysis of *X* chromosome (males)

	% effect or direction	Probability
Regions		
1-0.0 *y*	1	0.0746
1-4.1 *w*	None	0.4017
1-35.4 *m*	3	0.0650
1-56.0 *f*	7	0.0034
Interaction terms		
y * *w*	Negative	0.0020
y * *m*	None	0.7532
y * *f*	None	0.5199
w * *m*	None	0.7046
w * *f*	None	0.4798
m * *f*	None	0.6851

$N = 2200$ males.

Tables 4 and 5 show the results of the FUNCAT analysis for males and females. The difference between the resistance of the most *D. sechellia*-like *X* and the most *D. simulans*-like *X* is highly significant in both sexes. Furthermore, in both sexes, substitution of the *D. sechellia* region around *1*-56 significantly improves resistance. The region around *1*-35 has a borderline significant effect in males and a significant effect in females. Thus, there is at least one factor between *1*-35 and *1*-56. Similarly, the *D. sechellia* region near *1*-0 has a borderline significant effect in males and a significant effect in females.

In both sexes, the region around *1*-0 interacts negatively with the adjacent region of *1*-4.1. In females, there is a negative interaction between *1*-0 and *1*-56 as well. Although epistasis is clearly present, the cause of these interactions is not obvious.

TABLE 5

Recombination analysis of *X* chromosome (females)

	% effect or direction	Probability
Regions		
1-0.0 *y*	2	0.0277
1-4.1 *w*	None	0.3160
1-35.4 *m*	6	<0.0001
1-56.0 *f*	12	<0.0001
Interaction terms		
y * *w*	Negative	0.0035
y * *m*	None	0.6865
y * *f*	Positive	0.0290
w * *m*	None	0.1100
w * *f*	None	0.2869
m * *f*	None	0.8984

$N = 2355$ females.

TABLE 6

Recombination analysis of chromosome *3*

	% effect	Probability
Males		
3-19.2 *jv*	None	0.5054
3-46.3 *st*	18	<0.0001
3-59.4 *e*	21	<0.0001
3-68.6 *osp*	5	0.0329
3-97.3 *pe*	None	0.1390
Females		
3-19.2 *jv*	None	0.0972
3-46.3 *st*	9	0.0055
3-59.4 *e*	25	<0.0001
3-68.6 *osp*	18	<0.0001
3-97.3 *pe*	None	0.6277

$N = 1409$ males; $N = 1475$ females.

In sum, the *X* chromosome harbors a minimum of two factors affecting resistance: one between *1*-35 and *1*-56, and the other near *1*-0.

Chromosome *2*: To dissect chromosome *2*, I crossed *D. simulans nt* (*2*-0) *b* (*2*-45) *py* (*2*-74) *sd* (*2*-80) *pm* (*2*-108) females to *D. sechellia* line 1 males, backcrossed the F_1 females to the *D. simulans* parental strain, and tested the resistance of the resulting backcross progeny. As expected from the whole chromosome analysis, chromosome *2* has a slight effect on resistance. In females, there is an 11% difference in resistance between the most *D. sechellia*-like and most *D. simulans*-like genotypes (*D. sechellia*-like, 72% survival, $N = 333$; *D. simulans*-like, 61% survival, $N = 345$; $\chi^2 = 8.55$, $P = 0.0035$), while in males, there is a borderline significant 7% difference (*D. sechellia*-like, 69% survival, $N = 305$; *D. simulans*-like, 62% survival, $N = 234$; $\chi^2 = 3.044$, $P = 0.081$). Because these extreme genotypes showed such small differences, I had little power to map the factor(s) involved and thus did not pursue further mapping of this chromosome. Nonetheless, chromosome *2* must carry at least one resistance factor.

Chromosome *3*: The whole chromosome analysis showed that the third chromosome had the largest effect on resistance. Preliminary backcrosses dissecting the third were performed using a *jv st e pe* stock ($N = 1772$ females and 1257 males) and an *st e osp* stock ($N = 1513$ females and 1310 males). In the end, however, I repeated these analyses using a *D. simulans* stock bearing all five markers [*jv* (*3*-19.2) *st* (*3*-46.3) *e* (*3*-59.4) *osp* (*3*-68.6) *pe* (*3*-97.3)].

This backcross analysis revealed significant effects on resistance at *3*-46.3, *3*-59.4, and *3*-68.6 in both sexes (Table 6). This finding suggests that there is at least one resistance factor between *3*-46.3 and *3*-59.4 and one between *3*-59.4 and *3*-68.6. Table 6 shows that these factors near *3*-59.4 have the largest effect on resistance. Moreover, I detected the same very large effect near *3*-59.4 in both of my preliminary analyses of the third (*jv st e pe* experiment: $\chi^2 = 39.41$, $P < 0.0001$ for males, $\chi^2 = 64.15$, $P < 0.0001$ for females; *st e osp* experiment: $\chi^2 = 19.23$, $P < 0.0001$ for males, $\chi^2 = 32.90$, $P < 0.0001$ for females). In sum, I have strong evidence for a factor to the left of *3*-59.4 as well as for a factor to the right of *3*-59.4.

Neither the large region to the left of *3*-46.3 nor the large region to the right of *3*-68.6 has a discernible affect on resistance. Thus, at least 75% of the third chromosome appears to have no effect on octanoic acid resistance. Finally, no significant epistatic interactions were detected among any of the five markers.

In sum, most of the third chromosome's profound effect on resistance is due to (at least) two factors near *3*-59.4.

Chromosome *4*: Chromosome *4* comprises only 2% of the genome and does not recombine (Ashburner 1989). Thus the visible marker *eyeless (ey)* marks the entire chromosome. I crossed *D. simulans ey* females to *D. sechellia* line 1 males, backcrossed the F_1 females to *D. simulans ey*, and tested the resistance of the resulting progeny. Chromosome *4* has no effect on resistance in either males (wild type = 36% survival, $N = 231$; *ey* = 28% survival, $N = 225$; $\chi^2 = 3.294$, $P = 0.07$), or females (wild type = 47% survival, $N = 286$; *ey* = 43% survival, $N = 286$; $\chi^2 = 0.788$, $P = 0.37$).

DISCUSSION

Historically, two problems have frustrated genetic analyses of adaptation: a lack of genetic tools in species with unambiguous adaptations and a lack of unambiguous adaptations in species having abundant genetic tools. The *D. sechellia*/Morinda system overcomes these two problems. First, *D. sechellia* is clearly adapted to the otherwise-lethal effects of Morinda fruit. Second, it provides, along with its sister species, a large number of genetic tools (*e.g.*, mapped markers, compound chromosomes).

This study reveals that *D. sechellia*'s resistance to Morinda fruit toxin is dominant, that the factors affecting resistance reside on all the major chromosomes, and that there are some epistatic interactions among resistance factors. Chromosome *1* harbors at least two factors, one near *1*-0 and another between *1*-35 and *1*-56. Chromosome *2* carries at least one resistance factor, although the effect of this chromosome was too weak to allow further genetic dissection. Chromosome *3* has the largest effect on resistance. Two fairly small regions of large effect confer this resistance, one to the left of and one to the right of *3*-59.4. The remaining 75% of the chromosome has no discernible effect on resistance (see Figure 6). In sum, at least five genes are involved in *D. sechellia*'s resistance to octanoic acid.

This study begins to address two important issues in the genetics of adaptation. First, the present data allow

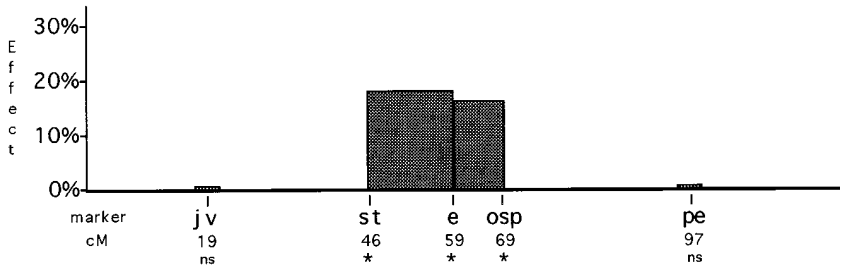

Figure 6.—Effect of introgressing regions of *D. sechellia* chromosome *3* into a mostly *D. simulans* background. These values are the average of male and female values (see Table 6) for these intervals. ns, no significant effect; *, significant effect.

me to eliminate the two extreme models for the genetic basis of *D. sechellia*'s adaptation: the single gene and infinitesimal models. Although the exact number of genes causing resistance remains unknown, my finding of small regions of large phenotypic effect *and* of large regions of no effect strongly suggests that resistance has an intermediate, "oligogenic" basis.

Several recent studies of morphological differences between species have also revealed such oligogenic bases. Bradshaw *et al.* (1995) showed that several differences in floral structure between species of Mimulus appeared due to a modest number of QTL of fairly large effect in each. Most of these traits are almost surely adaptive as they play a role in pollinator biology. True *et al.* (1997) showed that differences in male genitalia between two Drosophila species involved roughly 1–8 QTL of modest effect in each. These characters are very likely the products of sexual selection. Both studies also reported large chromosome regions of no apparent effect. Similarly, recent genetic analyses have shown that within-species resistance to parasites also has a simple, or at most oligogenic basis (Severson *et al.* 1995; Gorman *et al.* 1997; Orr and Irving 1997). These patterns are qualitatively similar to those characterizing the genetic basis of *D. sechellia*'s resistance to its host toxin. Although it is too early to offer any sweeping generalizations, these studies indicate that neither the infinitesimal nor the single gene model characterizes the genetics of natural adaptations. Instead, such adaptations typically may be oligogenic.

The present analysis allows us to address a second question: does the genetic architecture of resistance to a naturally occurring "pesticide" differ from that of resistance to agricultural pesticides? The selection pressures caused by natural pesticides probably differ from those caused by agricultural pesticides (which are often applied in very high concentrations over a brief time period). As noted earlier, resistance to agricultural pesticides typically involves one or two genes of large effect. Resistance to octanoic acid, on the other hand, involves at least five factors. The genetic basis of resistance to natural toxins may, therefore, be more complex than resistance to agricultural pesticides.

It is worth noting two limitations on the present study. First, these results tell us little about the biochemical basis of resistance. *D. sechellia*'s resistance to Morinda might involve either of two mechanisms: *D. sechellia* may carry genes that specifically detoxify octanoic acid and/or genes that confer a general stress response. This study does not allow us to distinguish between these biochemical possibilities. In either case, however, the alleles involved contribute to an obvious adaptation: resistance alleles (whether specific or general) are restricted to *D. sechellia*, and thus allow it to exploit a previously unavailable niche.

Second, like any analysis of between-species differences, this study tells us only about the genetic basis of current differences. It does not tell us about the chronology of substitutions involved in *D. sechellia*'s adaptation to Morinda. R'Kha *et al.* (1997), however, recently suggested a hypothetical evolutionary scenario describing *D. sechellia*'s specialization on Morinda. They proposed that *D. sechellia*'s susceptible generalist ancestor initially bred on rotten Morinda fruit, which is less toxic than ripe fruit. As this lineage gradually evolved greater resistance, it was able to exploit increasingly less rotten (and hence more toxic) Morinda, thereby gaining a temporal advantage over less-resistant competitors. This hypothesis is consistent with what is now known about the genetic architecture of resistance to octanoic acid. However, our finding of putative factors of large effect (for example, the small intervals to the left of and to the right of *3*-59.4) means that our results are *also* consistent with an alternative hypothesis: *D. sechellia* may have initially adapted to Morinda via one or two substitutions of large phenotypic effect followed by a series of substitutions of alleles of small effect at additional loci. To help distinguish between these scenarios, future experiments will (1) further dissect chromosome regions of large effect to determine if they contain single factors and (2) determine if any of these factors is sufficient—when placed alone in a susceptible *D. simulans* genome—for survival over an entire life cycle on ripe Morinda fruit.

Last, it is worth noting that *D. sechellia*'s adaptation to Morinda fruit may contribute to reproductive isolation between it and *D. simulans*. When *D. sechellia* was first collected on a few remote islands in the Seychelles, it was believed to be allopatric to *D. simulans* (Lachaise *et al.* 1988). However, populations of *D. simulans* have been found in remote locations on the main island, Mahé (although it is likely that *D. simulans* was introduced there during recent colonization by humans; Lachaise *et al.* 1988; R'Kha *et al.* 1991). Recently, popu-

lations of *D. sechellia* have also been found on Mahé (R'Kha *et al.* 1991). Given that these sympatric *D. simulans* and *D. sechellia* can produce fertile F_1 hybrid females, it is possible that *D. sechellia*'s preference for and resistance to Morinda acts as a form of prezygotic reproductive isolation between these species. If true, this study represents the first genetic dissection of ecologically based reproductive isolation (see Coyne and Orr 1998).

This is the first in a planned series of genetic studies of the *D. sechellia*/Morinda system. In future work, we plan to use microsatellite markers to further dissect the regions of large effect identified here. We have also begun to analyze two additional components of *D. sechellia*'s adaptation to its host plant: its larval resistance to, and oviposition-site preference for, Morinda fruit.

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