The genetic basis of larval resistance to a host plant toxin in *Drosophila sechellia*

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**Summary**

The larvae of *Drosophila sechellia* are highly resistant to octanoic acid, a toxin found in *D. sechellia*’s host plant, *Morinda citrifolia*. In contrast, close relatives of *D. sechellia*, *D. simulans* and *D. melanogaster*, are not resistant. In a series of interspecific backcrosses, 11 genetic markers were used to map factors affecting egg-to-adult (‘larval’) resistance in *D. sechellia*. The third chromosome harbours at least one partially dominant resistance factor. The second chromosome carries at least two mostly dominant resistance factors but no recessive factors. However, neither the X chromosome – which contains 20% of *D. sechellia*’s genome – nor the fourth chromosome appear to affect resistance. These data suggest that larval resistance to *Morinda* toxin may involve only a handful of genes. These results, when compared with a previous analysis of adult resistance to *Morinda* toxin in *D. sechellia*, suggest that larval resistance may involve a subset of the genes underlying adult resistance.

**1. Introduction**

The fruit of *Morinda citrifolia* is the only known host of *Drosophila sechellia* (Louis & David, 1986). In order to specialize on *Morinda, D. sechellia* has evolved resistance to and preference for *Morinda* fruit. In contrast, cosmopolitan sister species of *D. sechellia, D. melanogaster* and *D. simulans*, remain highly susceptible to the toxins in *Morinda* fruit and avoid it (R’Kha *et al*., 1991).

Adaptations to a particular host, like that seen in *D. sechellia*, are common among insects. Yet surprisingly little is understood about the genetic bases of such adaptations. In fact, very little is known about the genetics of any natural adaptation. Specifically, it is not known: (1) how many genes are typically involved in adaptation; (2) what distribution describes the phenotypic effects of adaptive alleles; or (3) what roles dominance and epistasis have in adaptation.

Population genetic theorists have laboured to answer these questions (reviewed in Orr & Coyne, 1992; Orr, 1998). Fisher, Kimura and others have proposed a variety of population genetic models of adaptation (Fisher, 1930; Robertson, 1967; Kimura, 1983; Lande, 1983; Bürger, 1993; MacNair, 1991). Unfortunately, as Orr & Coyne (1992) point out, these models are unsatisfactory as they either ignore important population genetic forces or require unrealistic assumptions.

Most data regarding the genetics of adaptation come from studies of adaptations to human disturbances, from agricultural research and from artificial selection experiments (reviewed in Orr & Coyne, 1992; Hoffmann *et al*., 1995; Kearsey & Farquhar, 1998). Unfortunately, most of these adaptations are responses to rapid and harsh environmental change caused by humans. ‘Natural’ adaptations, on the other hand, probably evolve over a longer period of time and may involve less severe selection. Thus the genetic basis of adaptation to human disturbances may tell us little about the genetic basis of natural adaptations.

Data on natural adaptations have been difficult to obtain. Historically, species showing unambiguous adaptations have often lacked the genetic tools required to analyse these phenotypes. Conversely, organisms with good genetic tools have often lacked striking adaptations. Fortunately, new genetic tools (such as polymorphic DNA markers) and new statistical tools (such as quantitative trait locus (QTL)
analysis) have opened many previously intractable species to genetic analysis. Bradshaw et al. (1998), for example, mapped QTLs underlying floral differences between two species of Monkeyflower (\textit{Mimulus}). Although several QTLs affect these traits, at least 25\% of the parental difference in each trait may be due to a single factor. Surprisingly, these adaptive floral differences are genetically neither very simple nor complex.

Several other studies have also suggested that adaptation may have a relatively simple genetic basis (Severson et al., 1995; Gorman et al., 1997; Orr & Irving, 1997; Voss & Shaffer, 1997; Hunt et al., 1998, 1999). While adaptation via single gene substitutions cannot always be ruled out (e.g. Voss & Shaffer, 1997; Orr & Irving, 1997), it is clear that adaptations are not as genetically complex as historically thought.

As part of its adaptation to \textit{Morinda}, adult \textit{D. sechellia} have evolved to be resistant to, and to oviposit on, ripe \textit{Morinda} fruit, which is toxic to other species of \textit{Drosophila} (Louis & David, 1986). An earlier study showed that resistance of adult \textit{D. sechellia} to \textit{Morinda} toxin is dominant in hybrids between \textit{D. sechellia} and \textit{D. simulans} and involves at least five genes (Jones, 1998). The two factors of largest effect map to two regions near the centromere of chromosome 3. Large chromosomal regions not affecting resistance were also found. These data suggest that the genetic basis of this adaptation is again neither very simple nor complex.

Although \textit{Morinda} fruit detoxifies as it rots (very rotten fruit can, in fact, be used by non-resistant species), \textit{D. sechellia} larvae have adapted to survive and develop in the normally inhospitable ripe fruit (Louis & David, 1986; Moreteau et al., 1994). R’Kha et al. (1991) showed that \textit{D. sechellia} larvae resist the toxic effects of \textit{Morinda} better than \textit{D. melanogaster}, \textit{D. mauritiana} or \textit{D. simulans} larvae. They also concluded that resistance is partially dominant in hybrids between \textit{D. simulans} and \textit{D. sechellia}. Later, Legal et al. (1992) discovered that the primary \textit{Morinda} fruit toxin is octanoic acid, a compound that represents 58\% of the fruit’s identifiable volatiles (Farine et al., 1996). (Hexanoic acid is also found in \textit{Morinda}, but in much smaller quantities and appears to be significantly less harmful (Legal et al., 1992).) Using pure octanoic acid, Amlou et al. (1998) repeated R’Kha et al.’s experiments, showing that \textit{D. sechellia} larvae also resist pure toxin. Neither R’Kha et al. nor Amlou et al., however, mapped the genes underlying resistance.

Here I investigate the genetics of egg-to-adult (‘larval’) resistance to the \textit{Morinda} toxin, octanoic acid, in \textit{D. sechellia}. In particular, I verify that \textit{D. sechellia} larvae are highly resistant, I measure the dominance of resistance in interspecific hybrids, and I recombination-map dominant and recessive factors affecting resistance.

2. Materials and methods

(i) Stocks

Stocks used are described in Table 1. All flies were reared at 24°C (±1°C) on agar–yeast–cornmeal medium.

(ii) Crosses

To estimate the dominance of resistance in F1 hybrids, susceptible \textit{D. simulans} Islamorada were reciprocally crossed to resistant \textit{D. sechellia} line 1 and the resistance (see below) of the resulting F1 male and female progeny was assayed.

To test for an effect of the X, crosses using compound-X chromosomes from \textit{D. simulans} were performed. First, \textit{D. simulans} \textit{C(1)RM}, \textit{yw} females were crossed to \textit{D. sechellia} line 1 males and the resistance of the resulting progeny was assayed. F1 males from this cross have an unrecombined X from \textit{D. sechellia} (and a \textit{D. simulans} Y), whereas females have both X chromosomes from \textit{D. simulans}. Second, to control for possible effects of the \textit{D. simulans} Y, the above cross was repeated using \textit{D. simulans} \textit{C(1)RM yw/C(1;Y) AB/0}. Males from this cross have an unrecombined \textit{D. sechellia} X but no Y.

To map dominant and partially dominant resistance factors, females from a multiply marked stock of \textit{D. simulans}, \textit{f}2 (1–56;0); \textit{nt} (2–0) \textit{pm} (2–103); \textit{st} (3–46;3) \textit{e} (3–59;4), were crossed to \textit{D. sechellia} line 1 males. The resulting F1 females were then backcrossed to males from the \textit{D. simulans} marker stock. This backcross produces 32 different genotypes. On average, three-quarter’s of the backcross progeny’s genes are derived from \textit{D. simulans} and one-quarter are derived from \textit{D. sechellia}. However, because recombination occurs in F1 females, markers do not identify the species origin of entire chromosomes. Nevertheless, markers do remain associated with large chromosomal regions. Mutant phenotypes indicate chromosome regions that are homozygous for material from \textit{D. simulans}, while wild-type phenotypes indicate chromosome regions that are heterozygous for \textit{D. sechellia} and \textit{D. simulans} material.

The visible marker eyeless, \textit{ey} (4–0), marks the entire chromosome 4 and was used to test the effect of chromosome 4. \textit{D. simulans} \textit{ey} females were crossed to \textit{D. sechellia} line 1 males. F1 females were then backcrossed to \textit{D. simulans} \textit{ey}.

To map recessive and partially recessive factors affecting resistance, multiply marked \textit{D. sechellia} females, \textit{zn} (1–25) \textit{f} (1–56); \textit{j} (2–48) \textit{cn} (2–67), were crossed to \textit{D. simulans} males bearing dominant markers (\textit{Ubx} (3–60)/\textit{Df}(3–66)). F1 females were then backcrossed to recessively marked \textit{D. sechellia} males. This backcross produces 32 different genotypes. The
resulting progeny are, on average, three-quarters *D. sechellia* and one-quarter *D. simulans*.

(iii) **Resistance assay**

Resistance to octanoic acid was measured by contrasting larval survival in media with octanoic acid to larval survival in media without octanoic acid. Specifically, equal numbers of inseminated females (*n* = 5) were placed in paired sets of control and toxic test chambers (each paired set of toxic and control chambers is a ‘paired replicate’). Chambers were 150 ml specimen cups with a cheesecloth lids. Each chamber contained about 30 ml of Drosophila Instant Medium (Carolina Biological Supply, Inc.). Toxic treatments were 0.07% (± 0.005%) octanoic acid by weight (Sigma Chemical Co.). This dose of toxin, while sufficient to differentiate susceptible from resistant larvae (see Section 3), does not kill the majority of ovipositing females. Furthermore, the toxic media became largely non-toxic by the time adult progeny emerged (Jones, 2000).

Females were allowed to oviposit for 3 days. A folded Kimwipe was placed in each chamber to give the larvae a place to pupate. The emerging adults were then counted and genotyped. All tests were performed at 24 °C (± 1 °C).

To account for genetic differences in egg production, the number of eggs laid was counted when comparing the survival of larvae having *D. sechellia* or *D. simulans* mothers and in pure species comparisons. Percentage survival was then calculated by dividing the number of emerging adults by the number of eggs laid in each chamber. The percentage survival in the toxic environment was then compared with that of the control environment.

This approach is unnecessary in backcross analyses as F1 females are genetically identical and, more importantly, the number of eggs of each genotype that were laid can not be known. Similarly, egg counts are not needed in compound-X crosses as it is the change in sex ratio – not survival per se – between the control and toxic treatments that is being assayed. In these experiments, the control treatment was used to determine how frequently each genotype (or sex in compound-X crosses) occurs among the progeny. This was compared with how frequently each genotype occurs among the progeny of the toxic treatment. If a particular genotype improves resistance, it will occur more frequently in toxic than in control treatments.

The above approach has two additional merits. First, because it estimates the frequencies of genotypes from control chambers, it controls for any deleterious effects of generic markers on viability. Second, it controls for any remaining bias caused by hybrid inviability.

(iv) **Statistics**

Frequency data from the backcrosses were analysed using the CATMOD procedure in SAS (for a complete description of this method see pp. 191–282 of SAS/STAT User’s Guide; SAS Institute, 1988). For markers *M*[1], *M*[2], …, *M*[n], the model statement was ‘MODEL RESISTANCE = *M*[1] *M*[2] … *M*[n] *M*[1]*M*[2] … *M*[1]*M*[n] … *M*[n-1]*M*[n].’ This procedure measures the main effects and interactions of a marker substitution by comparing all genotypes that differ in this substitution. The result is reported as a *χ*² statistic. For all other comparisons, contingency table analysis (reported as a *χ*²) or a *t*-test was used as appropriate (Statview, SAS Institute).

Percentage effects are the fraction of the difference in resistance between the most *D. sechellia*-like genotype and the most *D. simulans*-like genotype explained by a region of a chromosome in a backcross analysis.

The dominance of resistance in F1 hybrids (‘d’) was estimated by determining how much resistance in F1 hybrids deviated from the midparent mean resistance.
(Falconer & Mackay, 1996). Thus, ‘d’ ranged from −1 for complete recessives, to 0 for additivity, to 1 for complete dominants.

3. Results

(i) Interspecific comparison

Amlou et al. (1998) showed that larvae of sister species of D. sechellia are susceptible to the Morinda toxin, octanoic acid. They also showed that D. sechellia is highly resistant. This species difference was verified. Wild-type isofemale lines from D. melanogaster, D. simulans and D. sechellia were tested for resistance to octanoic acid. Not surprisingly, D. melanogaster and D. simulans both suffer in toxic media, whereas D. sechellia thrives (Table 2). This fact suggests that the common ancestor of D. sechellia and D. simulans was susceptible to octanoic acid.

(ii) Dominance of resistance

R’Kha et al. (1991) and Amlou et al. (1998) both suggested that resistance is not completely recessive, although these two studies disagreed on the degree of dominance. R’Kha et al., in a series of backcrosses, showed that larval resistance to the toxic effects of Morinda fruit is partially dominant. However, Amlou et al. noted that F1 hybrids between D. simulans females and D. sechellia males survive poorly when reared in media containing octanoic acid, suggesting that larval resistance is fairly recessive.

One possible explanation for the discrepancy between these earlier studies is that larval resistance may involve maternal effects. Even if resistance is dominant to susceptibility, F1 larvae must rely on maternal proteins during early development. Genotypically resistant F1 larvae may still suffer increased mortality if they have a susceptible mother, and resistance will appear mostly recessive. On the other hand, backcross progeny would not suffer from increased mortality because their F1 mothers are resistant, and resistance would appear partially dominant.

To test for a maternal effect, the larval resistances of reciprocal F1 hybrids were compared. In F1s with D. simulans mothers, larval resistance is partially recessive (d = −0.33 ± 0.18 SE; n = 177; 6 replicates). However, in F1s with D. sechellia mothers resistance is more dominant (d = 0.65 ± 0.14 SE; n = 87; 6 replicates). This difference is consistent with a maternal effect on resistance. (This effect is not due to larval density. Although D. simulans mothers lay 88% more eggs than D. sechellia mothers, the average percentage survival in control treatments for the offspring from D. simulans mothers is 36% greater than that of the offspring from D. sechellia mothers. This is the opposite of what is expected if increased density is negatively affecting viability.)

However, this apparent maternal effect result may be compromised by the fact that F1 males inherit their X chromosome from their mothers. If resistance involves X-linked genes, the above estimates of dominance would be misleading. To test for an effect of the X, several crosses using compound-X chromosomes from D. simulans were performed. F1 males from this cross have an unrecombined X from D. sechellia (and a D. simulans Y), whereas females have both X chromosomes from D. simulans. If the X has an effect on resistance, the sex ratio of F1s reared in the toxic treatment should be male-biased relative to that of F1s in the control treatment. It is not, which suggests that the X chromosome does not harbour resistance genes (males in control = 68%; males in toxic = 61%; n = 106; 4 replicates; χ² = 0.676; P = 0.411). Moreover, male fitness appears to decline in the toxic treatments relative to the controls (albeit not statistically significantly).

To control for possible effects of the D. simulans Y, the above cross was repeated using D. simulans C(1)RM yw /C(1)Y AB/0. Males from this cross have an unrecombined D. sechellia X but no Y. Again,
To map dominant larval resistance factors, a backcross to D. simulans does not harbour any resistance factors and, thus, these results suggest that the X chromosome probably effects of the X chromosome on resistance. Together – 3L 3 2R 0 – 3L 3 2R 0 males (see also Appendix, Tables A.2). The X chromosome does not. In both sexes the largest effect maps to the right arm of the third chromosome (3R) near 3–59. Similarly, the left arm of chromosome 2 (2L) affects resistance in both sexes. However, only male larvae show a significant effect of the right arm of chromosome 2 (2R). While females do not show a significant main effect of this region, they do show a significant positive interaction between this region and 3R.

(iii) Backcross to D. simulans

To map dominant larval resistance factors, a backcross was used to introduce regions of D. sechellia chromosomes into an otherwise D. simulans genome.

Tables 3 and 4 show that chromosomes 2 and 3 carry factors affecting resistance in both females and males (see also Appendix, Tables A.1 and A.2). The X chromosome does not. In both sexes the largest effect maps to the right arm of the third chromosome (3R) near 3–59. Similarly, the left arm of chromosome 2 (2L) affects resistance in both sexes. However, only male larvae show a significant effect of the right arm of chromosome 2 (2R). While females do not show a significant main effect of this region, they do show a significant positive interaction between this region and 3R.

Finally, the backcrosses detailed below rule out an effect of the X chromosome on resistance. Together these results suggest that the X chromosome probably does not harbour any resistance factors and, thus, show that resistance involves a maternal effect.

The effect of chromosome 4 on resistance was also tested. This chromosome represents less than 2% of the genome and does not recombine (Hochman, 1976). Table 5 shows that chromosome 4 does not harbour dominant factors affecting resistance.

A few problems potentially complicate the present analysis. First, because F1 hybrid females were used, recombination reduces the chance that resistance factors that are loosely linked to markers will be detected. This problem is most serious near the center of chromosome 2 and the distal ends of chromosome 3. As a result, the effects of resistance factors in these regions will be underestimated. This may explain why the effects of detected factors do not sum to 100% (see Tables 3, 4).

Second, because interspecific hybrids were used, it is possible that hybrid incompatibilities – if they are worsened in the toxic environment – may obscure the effects of resistance factors. Although there is no direct evidence for this kind of interaction, it cannot be ruled out.

Finally, and most importantly, the above crosses cannot identify mostly recessive resistance factors. To solve this problem, the reciprocal backcross was performed.

(iv) Backcross to D. sechellia

To identify chromosomal regions harbouring recessive genes affecting larval resistance, D. simulans chromosomes were moved into an otherwise D. sechellia genome.

CATMOD analysis showed that only the region near 3–60 significantly affects resistance in females (n = 1177; 21 replicates; $\chi^2 = 12.06; P = 0.0005$; see Appendix Table A.3). In males, this effect is of borderline significance, but this may, in part, reflect a smaller sample size (n = 1075; 21 replicates; $\chi^2 = 3.75; P = 0.0528$; see Appendix Table A.4). In both sexes, no other chromosomes have significant effects. In females, however, there is also a significant positive interaction between 2L and 2R ($\chi^2 = 5.52, P = 0.0188$).

For increased statistical power, female and male data were pooled and the CATMOD analysis re-
peated. Again, 3–60 had a significant effect on resistance \((n = 2256; 21 \text{ replicates}: \chi^2 = 13.46; P = 0.0002)\), and again there was a positive interaction between 2L and 2R \((\chi^2 = 4.66; P = 0.0309)\). However, no other regions appear to harbour resistance factors. The factor linked to 3–60 explains 29% of the difference in resistance between the most \(D. sechellia\)-like genotype and the most \(D. simulans\)-like genotype.

Again, recombination in F1 hybrid females reduces the ability to detect resistance factors, especially on the distal regions of chromosome 2 and on the left arm of chromosome 3. Thus the above estimate of the effect of the factor near 3–60 is probably an underestimate.

4. Discussion

This study identifies \(D. sechellia\) chromosome regions affecting egg-to-adult (‘larval’) resistance to the \(Morinda\) toxin, octanoic acid. Resistance is partially dominant and is controlled by a minimum of three genes: one on chromosome 3 and two on chromosome 2. The X and fourth chromosomes have no detectable effect on resistance.

The backcross to \(D. simulans\) suggested that the second and third chromosomes harbour dominant or partially dominant factors affecting resistance. The effect of chromosome 3 on resistance is greater than that of the second chromosome and involves at least one resistance factor, near 3–60.

The backcross to \(D. sechellia\) shows that chromosome 3 may also harbour at least one partially recessive resistance factor. However, the markers on the third chromosome used in the dominant and recessive analyses were only 1 cM apart. This suggests, but does not prove, that the factor on this chromosome may be a single gene with an additive effect.

On the second chromosome, the backcross to \(D. simulans\) shows that the left arm affects resistance in both males and females. In males, the right arm of the second chromosome also affects resistance. In females, this region only affects resistance when the factor of large effect on chromosome 3 is also present. This interaction is not significant in males. More work is needed to determine whether the factor on 2R found in males is the same as the factor involved in the between-chromosome interaction in females.

Although chromosome 2 harbours at least one dominant resistance factor, no recessive factors were found on this chromosome. However, because the markers used in the recessive analysis are not near the markers used in the dominant analysis, a weak recessive effect of the dominant factor cannot be ruled out.

The X chromosome appears not to harbour any resistance factors. This was shown in the two compound-X crosses and in both backcrosses. While it is formally possible that one or a few genes of very weak effect could reside on this chromosome, it is clear that the sum of their effects must be considerably less than the 20% expected under a polygenic model.

Sex-specific effects occurred in both backcrosses. On chromosome 3, the backcross to \(D. sechellia\) suggests that the recessively acting effect of 3–60 is weaker in males. Chromosome 2 showed sex-specific effects in both backcrosses. The backcross to \(D. simulans\) suggests that 2R affects resistance in both sexes. In males this is a main effect, whereas in females this is an interaction. The backcross to \(D. sechellia\), also showed a significant interaction on chromosome 2 in females. Unfortunately, the genetic mechanism of these differences is not known. A number of other QTL studies have shown similar sex-specific effects (e.g. Vieira et al., 2000), but little is understood about the genes or mechanisms underlying these effects.

In sum, these data show that larval resistance involves a minimum of three dominant or semi-dominant genes. More importantly, they also show that large regions of \(D. sechellia\)'s genome probably do not harbour any resistance factors. For example, the X chromosome – which represents 20% of the genome of \(D. sechellia\) – has no effect on resistance. These results suggest that larval resistance does not involve a large number of genes, although the actual number of genes involved remains unknown.

The above result is consistent with a prior analysis of adult resistance in \(D. sechellia\) as well as with several other studies of natural adaptations (Severson et al., 1995; Gorman et al., 1997; Orr & Irving, 1997; Voss & Shaffer, 1997; Bradshaw et al., 1998; Hunt et al., 1998, 1999; Jones, 1998). Together, these data suggest that two extreme views of the genetics of adaptation can be ruled out: the single gene model and Fisher’s ‘infinitesimal’ model.

Despite the fact that we know what the genetic basis of adaptation is not, we still have a limited understanding of what the genetic basis of adaptation is. Theoretical work suggests that the phenotypic effects of genes underlying adaptation towards a fixed phenotypic optimum should be approximately exponentially distributed (Robertson, 1967; Orr, 1998, 1999). Thus, some of the genes involved in an adaptation should have moderate to large phenotypic effects. So far, the experimental data are consistent with this idea: several studies have found small chromosomal regions harbouring factor(s) of large phenotypic effect (Severson et al., 1995; Gorman et al., 1997; Orr & Irving, 1997; Bradshaw et al., 1998; Hunt et al., 1998, 1999). Nonetheless, this interpretation must remain tentative until finer-scale mapping can determine whether these factors of large effect reflect the action of one or several genes.

Likewise little is known about the mechanism of octanoic acid resistance, making it difficult to identify
candidate resistance genes. Physiological studies may be able to determine whether octanoic acid is effluxed like other cytotoxic compounds. However, until the individual genes affecting *D. sechellia*’s resistance are identified, we can conclude little about the genetic changes responsible for resistance.

The data are at least suggestive about the relationship between the genes involved in adult and larval resistance. The backcross to *D. simulans* is comparable to an earlier analysis of adult resistance (Jones, 1998). Indeed the same genetic tools and stocks were used both analyses. Contrasting these studies will show whether the genetics of larval and adult resistance are similar.

In both cases, the third chromosome has the largest effect on resistance. In larvae, this effect is due to a partially dominant resistance factor near 3–60. An adult resistance factor resides in this same region (Jones, 1998). This suggests that adults and larvae may share the resistance factor to the right of 3–60. However, an additional adult resistance factor resides to the left, linked to 3–46, a region having no effect in larvae. This strongly suggests that the genetics of adult and larval resistance are not identical.

Like the third, the second chromosome also harbours both adult and larval resistance factors. However, because adult resistance factors were too weak to be precisely mapped on this chromosome, it was not possible to determine whether adult resistance factors reside in the same regions as the larval resistance factors.

The data from the X chromosome, however, prove that the genetics of adult and larval resistance are not identical. In adults, the X chromosome has a greater effect on resistance than chromosome 2, whereas, in
Table A.3. Female larval resistance data from the backcross to D. sechellia

| Genotype       | Toxic treatment | Control treatment | Relative survival (T/C) |
|----------------|-----------------|-------------------|------------------------|
| + +; + +; Ubx  | 24              | 60                | 40%                    |
| + +; + +; +    | 22              | 55                | 40%                    |
| + +; + + cn; Ubx | 4              | 13                | 31%                    |
| + +; + cn; +   | 9               | 19                | 47%                    |
| + +; j +; Ubx  | 7               | 15                | 13%                    |
| + +; j + cn; Ubx | 10             | 13                | 46%                    |
| + +; j + cn; + | 29              | 52                | 56%                    |
| + +; j cn; +   | 30              | 40                | 75%                    |
| + f; + +; Ubx  | 6               | 25                | 24%                    |
| + f; + + cn; Ubx | 5              | 10                | 50%                    |
| + f; + + cn; + | 6               | 9                 | 67%                    |
| + f; j +; Ubx  | 1               | 1                 | 100%                   |
| + f; j + cn; Ubx | 11             | 27                | 41%                    |
| + f; j cn; +   | 17              | 19                | 89%                    |
| zn + + + +; Ubx | 15             | 43                | 35%                    |
| zn + + + + cn; Ubx | 16             | 33                | 48%                    |
| zn + + + +; +  | 3               | 11                | 27%                    |
| zn + + + + cn; + | 3              | 7                 | 43%                    |
| zn + + j +; Ubx | 3               | 6                 | 50%                    |
| zn + + j + cn; Ubx | 7             | 21                | 33%                    |
| zn + + j cn; + | 11              | 11                | 100%                   |
| zn f + + Ubx    | 34              | 57                | 60%                    |
| zn f + + + + Ubx | 32             | 49                | 65%                    |
| zn f + + cn Ubx | 2               | 8                 | 25%                    |
| zn f + + cn; + | 4               | 11                | 36%                    |
| zn f ; j +; +  | 3               | 12                | 25%                    |
| zn f ; j + Ubx  | 4               | 14                | 29%                    |
| zn f ; j cn; Ubx | 18             | 46                | 39%                    |
| zn f ; j cn; +  | 35              | 45                | 78%                    |

Table A.4. Male larval resistance data from backcross to D. sechellia

| Genotype       | Toxic treatment | Control treatment | Relative survival (T/C) |
|----------------|-----------------|-------------------|------------------------|
| + +; + +; Ubx  | 29              | 47                | 62%                    |
| + +; + +; +    | 36              | 42                | 86%                    |
| + +; + + cn; Ubx | 5              | 12                | 42%                    |
| + +; + cn; +   | 7               | 12                | 58%                    |
| + +; j +; Ubx  | 5               | 7                 | 71%                    |
| + +; j + cn; Ubx | 10            | 19                | 53%                    |
| + +; j cn; +   | 20              | 30                | 67%                    |
| + +; j cn; +   | 34              | 39                | 87%                    |
| + f; + +; Ubx  | 14              | 19                | 74%                    |
| + f; + + cn; Ubx | 2              | 4                 | 50%                    |
| + f; + + cn; + | 4               | 3                 | 133%                   |
| + f; j +; Ubx  | 5               | 6                 | 83%                    |
| + f; j + cn; Ubx | 9              | 25                | 36%                    |
| + f; j cn; +   | 18              | 30                | 60%                    |
| zn + + + +; Ubx | 16             | 26                | 62%                    |
| zn + + + + cn; Ubx | 20            | 39                | 51%                    |
| zn + + + +; +  | 5               | 5                 | 100%                   |
| zn + + + + cn; + | 4              | 6                 | 67%                    |
| zn + + j +; Ubx | 3               | 8                 | 38%                    |
| zn + + j + cn; Ubx | 4             | 5                 | 80%                    |
| zn + + j cn; Ubx | 11             | 13                | 85%                    |
| zn + + j cn; + | 14              | 16                | 88%                    |
| zn f ; + + Ubx  | 21              | 43                | 49%                    |
| zn f ; + + + + Ubx | 21            | 58                | 36%                    |
| zn f ; + cn Ubx | 3               | 9                 | 33%                    |
| zn f ; + cn; + | 6               | 14                | 43%                    |
| zn f ; j +; +  | 5               | 4                 | 125%                   |
| zn f ; j + Ubx  | 4               | 12                | 33%                    |
| zn f ; j cn; Ubx | 16             | 40                | 40%                    |
| zn f ; j cn; +  | 38              | 26                | 146%                   |

larvae, the X has no effect on resistance. Clearly, the genetics of larval and adult resistance are different even though larvae and adults may share some resistance genes. This type of result is not new. Some of the genes conferring larval resistance to DDT in mosquitoes and *Drosophila*, for instance, differ from those conferring adult resistance (Ford, 1971; Lindsley & Zimm, 1992), or these resistance genes have different effects at different life stages (e.g. *Rst*(2)*DDT*; Lindsley & Zimm, 1992).

Because *D. simulans* is believed to be sympatric with *D. sechellia* on some of the Seychelles Islands (R’Kha et al., 1997), *D. sechellia*'s specialization on *Morinda* may contribute to reproductive isolation between these species. If so, the present genetic analysis also suggests that the genetics of ecological isolation is fairly simple.

Unfortunately, little is know about how resistance evolved in *D. sechellia*. Given that there is a maternal effect on larval survival, it would be interesting to know how much selection for larval resistance affected the evolution of adult resistance and vice versa. Moreover, not much is know about the ecological forces that drove host specialization in *D. sechellia*: was it driven by competition from other species, or by predation from parasitoids, etc. Further genetic and ecological work is required to address these issues.

Although renewed interest in the genetics of adaptation has produced much-needed new data, our understanding of the genetics of adaptation remains rudimentary. We still do not know the types of genes that change during the evolution of an adaptation. Nor do we know what types of mutations are typically involved in adaptation. Answering these questions demands that the genes underlying adaptation be precisely mapped and identified. *D. sechellia*'s adaptation to *Morinda* provides a perfect opportunity for such analysis.
Appendix

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