# The Competitive Ability and Fitness Components of the *Methoprene-tolerant* (*Met*) Drosophila Mutant Resistant to Juvenile Hormone Analog Insecticides

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ABSTRACT

The *Methoprene-tolerant* (*Met*) mutation of *Drosophila melanogaster* results in a high (100-fold) level of resistance to the insecticide methoprene, a chemical analog of juvenile hormone. Pest species that are under control with methoprene may therefore have the potential to evolve resistance via a mutation homologous to *Met*. To evaluate the potential of such mutants to persist in wild populations, we must understand the fitness of flies carrying *Met*. In the absence of methoprene, *Met* flies were outcompeted by a wild-type strain both in a multigeneration population cage and in single-generation competition experiments. To determine which fitness component(s) is responsible for the competitive disadvantage, the survival, time of development, and fecundity of flies homozygous for each of five *Met* alleles were compared with wild type. Small but significant differences were found between the pooled *Met* alleles and wild type for pupal development time, pupal mortality, and early adult fecundity. These differences result in a large competitive disadvantage. Although *Met* flies were found to have reduced fitness by these measures, the phenotype is not as severe as might be expected from a knowledge of the disruption of juvenile hormone regulation seen in *Met* flies. It is concluded that (1) although *Met* flies have a large advantage under methoprene selection, they will quickly become outcompeted upon relaxation of methoprene usage, (2) even a seemingly severe disruption of juvenile hormone regulation has no drastic effect on the vital functions of the insect and (3) small differences in fitness components can translate into a large competitive disadvantage.

WHEN a susceptible population of insects is placed under insecticidal selection pressure, the evolution of insecticide resistance is a nearly certain event (GEORGHIOU and SAITO 1983). Indeed, resistance to insecticides remains a major problem in the management of agriculturally and medically important insects (METCALF 1983; GEORGHIOU 1986). A resistant population arises presumably via the replacement of susceptible wild-type individuals with one or more insecticide-resistant variants in the population. These variant insects carry at least one resistance gene, which is an allele of a wild-type gene. These resistance genes are thought to be uncommon in susceptible populations (CROW 1957). The rate of spread of a resistance gene in a population depends on a number of factors, including selection pressure, immigration of susceptibles into the population and the relative fitness of individuals carrying the gene (GEORGHIOU and TAYLOR 1977; MAY and DOBSON 1986).

Although the rate of spread of a resistance gene through a population is a paramount issue, how rapidly the population reverts to susceptibility when the selection pressure is relaxed is of almost equal importance. The rate of reversion will determine the success of alternation of insecticides, an agricultural practice designed to delay the spread of a resistance gene(s) in a population (METCALF 1980). The reversion rate depends to a large extent on the relative fitness of the resistance gene in the absence of insecticide. Obviously, a resistance gene will be more easily managed if it results in poor fitness than if it results in high fitness.

A number of studies have measured the fitness of insects that are resistant to a variety of insecticides (reviewed in ROUSH and MCKENZIE 1987), either by examining the components of fitness or by placing resistant insects in competition with susceptible ones. A survey of studies measuring various fitness components of different resistant strains led ROUSH and MCKENZIE to conclude that many, perhaps most, resistant strains had surprisingly high fitness relative to susceptible ones. As an extreme example, BEEMAN and NANIS (1986) found alleles of a malathion-resistant Tribolium gene to result in essentially wild-type fitness, as judged by competition studies with wild-type individuals.

We have used *Drosophila melanogaster* to study resistance to methoprene, a chemical analog of juvenile hormone (JH) that is widely used as an insect growth regulator insecticide (STAAL 1975). A locus, *Metho-*

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*prene-tolerant* (*Met*), has been identified, and an ethyl methanesulfonate (EMS)-induced allele, *Met*, has been genetically characterized (WILSON and FABIAN 1986). In this paper we will refer to this allele as $Met^1$ to distinguish it from the generalized *Met* mutant genotype. Flies homozygous for $Met^1$ show high resistance to several JH analog insecticides (WILSON and FABIAN 1986; RIDDIFORD and ASHBURNER 1991) as well as to JH III, one of two naturally occurring JH molecules in Drosophila (SLITER *et al.* 1987; BOWNES and REMBOLD 1987). A number of different alleles of *Met* have been isolated using different mutagenizing agents (WILSON and FABIAN 1987; T. G. WILSON, unpublished). The biochemical basis of *Met* resistance results from an altered cytosolic JH binding protein with reduced sensitivity to JH (SHEMSHEDINI and WILSON 1990), a type of resistance termed target-site resistance (KNIPPLE, BLOOMQUIST and SODERLUND 1988). This JH binding protein is believed to be a JH receptor (SHEMSHEDINI, LANOUE and WILSON 1990). In summary, the genetics and biochemistry are relatively well understood for this resistance gene.

Since *Met* influences a basic endocrine mechanism, it seems likely that other insects have a $Met^+$ homolog and thus the potential for resistance via this gene. Therefore, determining the relative fitness of flies carrying *Met* is an important issue for predicting the persistence of *Met* homologs in other insect populations.

A second reason for examining *Met* fitness centers on the role of JH in these flies. A paradox exists in that *Met* alters a putative JH receptor, a result expected to cause a severe depression in one or more fitness components since this hormone is involved in insect development and reproduction (RIDDIFORD 1985). Indeed, this expectation led WILLIAMS (1967) to predict that resistance would not develop to JH-analog insecticides, since any mechanism leading to resistance to a JH analog might be expected to result in an inability of the insect to regulate its own JH titer. Yet in initial studies (WILSON and FABIAN 1986; 1987) $Met^1$ flies were shown to have both high resistance to methoprene or JH III and unexpectedly high fecundity. A careful study of the fitness of *Met* flies might shed light on this paradox and possibly on the roles of JH in these flies.

## MATERIALS AND METHODS

**Drosophila stocks:** The Oregon-RC, *vermilion* (*v*), and *yellow vermilion* (*y v*) stocks were obtained from the Indiana University Collection and are described by LINDSLEY and ZIMM (1990). The attached-autosome stock, *C(3L)RM, ri/ C(3R)RM, sr*, from the Bowling Green State University collection, is similar to attached-autosomal stocks described by JUNGEN and HARTL (1979) and HAYMER and HARTL (1982). Flies were raised on a standard agar-yeast-molasses-cornmeal diet. While nutritionally adequate, this diet was not optimal. This is evident not only by the longer-than-average developmental times observed during this study but also by the increased fecundity that can be observed upon addition of baker's yeast to the adult diet (T. G. WILSON, unpublished observations). To ensure uniformity of diet, none of the diets in this study were supplemented with yeast. Care was taken to prevent overcrowding of the larvae in each culture. All flies were maintained on a 12:12 light:dark photoperiod. All cultures except the egg hatch determinations were maintained at 23 ± 1°.

**Mutagenesis:** The *Met* alleles used in this study were generated from Oregon-RC, *v* or *y v* males treated with either EMS by the procedure of LEWIS and BACHER (1968) or $^{60}Co$ irradiation (3000 rad). $F_1$ progeny from the mutagenized males were screened for *Met* alleles by the procedure of WILSON and FABIAN (1987). Resistant flies carrying putative *Met* alleles were crossed with the *FM7* balancer to create lines for additional testing of fertility and allelism with *Met*. This included tests with two deficiency chromosomes, $Df(1)m^{259\text{-}4}$ and *Df(1)N71*, that uncovered $Met^1$ and resulted in methoprene resistance (WILSON and FABIAN 1986). In addition, flies carrying each of the EMS-induced alleles have been biochemically characterized as having an altered JH binding protein (SHEMSHEDINI and WILSON 1990). Thus, each of the methoprene-resistant stocks examined in this study clearly carry *Met* alleles, and the resistance probably results entirely from the *Met* allele. Each allele has been maintained in homozygous condition in the absence of methoprene since its recovery during the past six years. Each is retested periodically with methoprene, and no loss of resistance has been evident.

**Fitness values:** Egg hatch at 25 ± 1° was determined 24 hr after allowing females to oviposit over a 4-hr period under uncrowded conditions.

For determination of both larval and pupal mortality and developmental times, larvae were transferred within 4 hr of hatching to a 9-dram glass shell vial containing food at a density of 50 larvae per vial. Larval developmental times were measured by recording time to pupariation at 12-hr intervals, and mortality determined by the number of pupae formed in each vial. Similarly, pupal developmental times were measured by recording eclosion at 12-hr intervals, and pupal mortality from the number of adults eclosing.

We were especially interested in any changes in the length of larval development not only as a measure of relative fitness but also as a possible indicator of the role of JH in dipteran larval development. JH has been shown to modulate larval development (hence the name juvenile hormone) in certain other insects such as Lepidoptera and Hemiptera (RIDDIFORD 1985). In these insects experimental withdrawal of JH during larval development can lead to premature metamorphosis (NIJHOUT and WHEELER 1982), and excess JH just prior to pupation results in delayed metamorphosis (HAMMOCK *et al.* 1990). However, no such dramatic influence of JH has been shown for dipteran insects. This is partially due to experimental difficulties in abating either JH or JH reception in these insects. Since *Met* flies have a less sensitive putative JH receptor (SHEMSHEDINI and WILSON 1990), then a role for JH in Drosophila larval development might be reflected as reduced larval developmental time.

Adult mortality was measured daily from adults isolated from culture bottles 0–24 hr after eclosion and placed in 9-dram food vials at an initial density of 20 males and 20 females per vial. Food was changed every other day. Survival was recorded until 90% of the adults in each vial were dead.

Fecundity was determined from females isolated from culture bottles 0–24 hr after eclosion and placed in food vials with an equal number of males of the same genotype

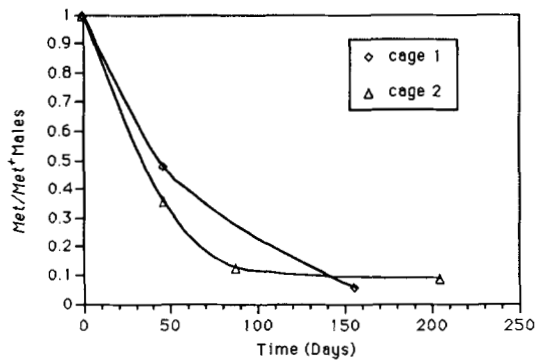


FIGURE 1.—Competition between *Met* and wild-type (Oregon-RC) Drosophila in a Sved population cage. Each point is the ratio of genotypes measured from a sample of 50 males obtained from each cage at the indicated time after cage initiation. Cage 1 and cage 2 represent duplicate experiments.

at a density of 20 females per vial. A larger vial having a food surface area of 6.2 $cm^2$ was used in these studies. Food was changed daily.

**Competition:** Multigeneration competition was carried out in plastic cages fitted with a gloved entry using the procedures described by SVED (1975), slightly modified by the addition of a vial containing wet cotton to provide added moisture for the flies. Each cage was initiated with 100 adults (nonvirgins) of each sex of both Oregon-RC and $Met^1$. At periodic intervals of about 50 days an extra food vial was placed in each cage to obtain an egg collection. Adult males were isolated from the culture of these eggs and tested for the $Met^1$ allele by crossing each with two attached-*X* females and raising the progeny on food supplemented with 5 nl/vial of methoprene. Under these conditions, the only progeny to eclose are $Met^1$ males, thus permitting unambiguous scoring of the genotype of the patroclinous *X* chromosome. The methoprene used was ZR-2008, obtained from Zoecon Corporation, Palo Alto, California.

Single-generation competition experiments were carried out according to the procedure of HAYMER and HARTL (1982). Bottles were initiated with 25 virgin adults of each sex of the attached-autosome stock and the competitor strain. The adults were transferred to fresh bottles after 3 days, and an additional 3-day oviposition was obtained. All progeny from each bottle were raised to adults and scored for genotype.

Comparisons were made using analysis of variance (ANOVA). Data reported as percentages and proportions were subjected to arc-sine transformation.

## RESULTS

**Competition between *Met* and wild type:** Initially, we determined the ability of $Met^1$ flies to compete with a laboratory wild-type strain, Oregon-RC, in a SVED (1975) population cage. Two cages were initiated with equal numbers of $Met^1$ and Oregon-RC flies. At periodic intervals during cage development samples of eggs were withdrawn, raised to adults, and a portion of the adult males tested for the presence of $Met^1$ or $Met^+$. As can be seen in Figure 1, $Met^1$ males were rapidly displaced in both populations by wild-type males to a low frequency in each population. Therefore, it seems that the $Met^1$ mutant has a fitness disadvantage relative to Oregon-RC flies under these conditions.

This inability of $Met^1$ flies to compete with wild-type could be due to either the $Met^1$ gene or to another gene(s) in the background genome of the $Met^1$ strain. To distinguish between these possibilities, we examined strains homozygous for five *Met* alleles that had been recovered from several different Drosophila strains during the course of our work on *Met*. These *Met* alleles were recovered following mutagenesis by either EMS or X-rays, and the strength of each allele as measured by resistance to methoprene was determined (Table 1). The background genome was not constant among these strains, and no effort was made to introduce the alleles into an isogenic background. Thus, this collection of alleles mimics a field population in which *Met* mutations arise independently and become established in flies having different background genomes.

The competitiveness of each of these strains against wild type was evaluated using the attached-autosome procedure described by HAYMER and HARTL (1982). In this method, equal numbers of virgin male and female competitor flies (either wild type or one of the *Met* alleles) are placed with the same number of virgin attached-autosomal (AA) flies, which are genetically marked with the *radius interruptus* (*ri*) and *stripe* (*sr*) mutations. All matings between the competitor and AA flies result in no viable progeny; thus, the two genotypes are reproductively isolated. Examination of the ratio of competitor to AA in the progeny thus provides a measure of the competitive ability of *Met* flies relative to wild type.

The results are shown in Figure 2. A comparison between pooled *Met* strains and Oregon-RC was significantly different ($F = 6.31$, $P = 0.023$). Thus, it is

TABLE 1

**Survival or pupae carrying *Met* alleles on various concentrations of methoprene**

| Genotype | Survival (%) with concentration of methoprene (μl/vial) 0 | 0.005 | 0.01 | 0.05 | Origin | Mutagen |
|---|---|---|---|---|---|---|
| *Met/Y* | 94 | 87 | 57 | 0 | Oregon-RC | EMS |
| *Met/Met* | 89 | 90 | 22 | 17 | Oregon-RC | EMS |
| $Met^2/Y$ | 91 | 64 | 30 | 10 | Oregon-RC | EMS |
| $Met^2/Met^2$ | 93 | 53 | 5 | 0 | Oregon-RC | EMS |
| $Met^3/Y$ | 73 | 60 | 53 | 36 | *y v* | EMS |
| $Met^3/Met^3$ | 91 | 80 | 52 | 12 | *y v* | EMS |
| $Met^{D29}/Y$ | 94 | 84 | 73 | 18 | *v* | γ-Rays |
| $Met^{D29}/Met^{D29}$ | 95 | 98 | 75 | 14 | *v* | γ-Rays |
| $Met^{N6}/Y$ | 74 | 71 | 48 | 23 | *v* | γ-Rays |
| $Met^{N6}/Met^{N6}$ | 65 | 91 | 64 | 0 | *v* | γ-Rays |
| Oregon-RC males | 86 | 0 | 0 | 0 | | |
| Oregon-RC females | 92 | 0 | 0 | 0 | | |

Each value is the average pupal survival of duplicate determinations taken from separate cultures. *Y* refers to the *Y* chromosome.

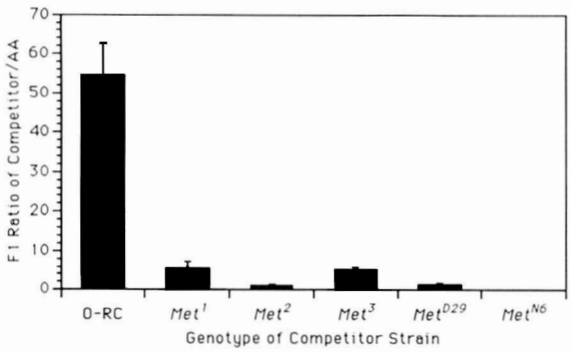


FIGURE 2.—Single-generation competition between either *Met* or Oregon-RC flies (competitor strains) and an attached-autosomal strain (indicated as AA). Each value is the mean ± SEM of the ratio of competitor to AA progeny produced in triplicate pairs of cultures of each strain ("competitor") in competition with AA. The value for $Met^{N6}$ is 0.12 ± 0.06, too small to appear in the figure. Although the results are presented as ratios, the data were calculated as proportions for statistical comparison (SOKAL and ROHLF 1981).

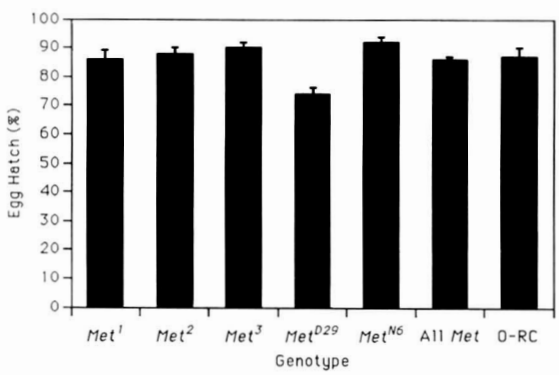


FIGURE 3.—Percentage egg hatch for various *Met* and Oregon-RC strains. Each determination is the mean ± SEM hatch recorded 24 hr after oviposition of 25 eggs by females of the indicated genotype. Each strain was replicated 8–12 times.

clear that none of the flies carrying *Met* alleles approach wild type in their ability to compete with the AA strain. Since the common feature of the *Met* flies is the presence of *Met*, it appears that this mutant gene confers a competitive disadvantage.

**Components of fitness:** The reason(s) for the competitive disadvantage of *Met* may possibly be identified by examining and comparing the components of fitness of the *Met* alleles relative to wild type. Although no conclusion can be drawn from the comparison of any one *Met* strain with wild type, similar results in all of the *Met* strains would suggest an effect of the *Met* gene on that component. The fitness components that were examined included egg hatch, mortality at each stage in development, length of each stage of development, and fecundity. Although this is not an exhaustive list of fitness components, certainly they would be major influences on the competitive ability of *Met* flies.

*Egg hatch:* The percentage egg hatch for the *Met* strains together with wild type is shown in Figure 3. The pooled *Met* had a slightly lower egg hatch than

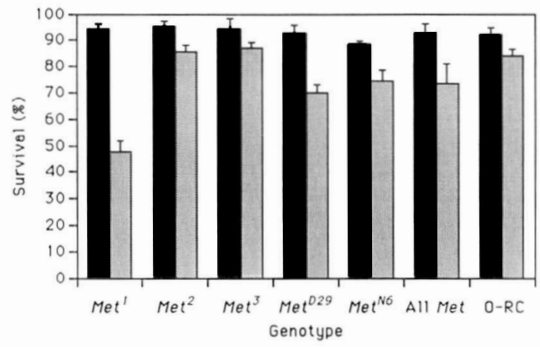


FIGURE 4.—Survival of larvae (■) and pupae (□) of various *Met* and Oregon-RC strains. Each determination is the mean ± SEM for 8–10 replicates of 50 larvae per vial.

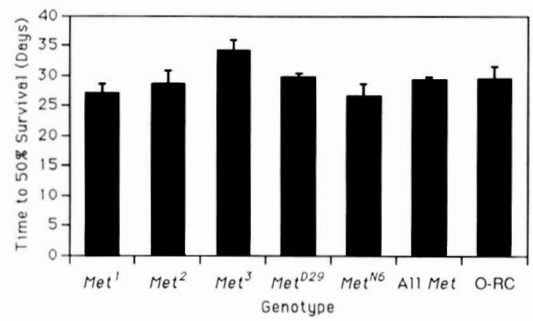


FIGURE 5.—Adult survival for various *Met* and Oregon-RC strains. Each determination is the mean ± SEM number of days after eclosion until 50% mortality was reached for each culture, replicated four times.

wild type, but the difference was not significant ($F = 0.052$, $P = 0.82$). Therefore, embryonic development does not seem to be affected in *Met* flies.

*Larval survival:* When survival during larval development was measured (Figure 4), no significant difference between wild type and pooled *Met* was evident ($F = 1.00$, $P = 0.94$).

*Pupal survival:* Survival of the pupae that developed in the above vials was measured (Figure 4). Significant differences were found among the six strains ($F = 13.82$, $P < 0.001$), and a comparison between wild type and pooled *Met* strains was found to be marginally significant ($F = 4.01$, $P = 0.05$). Examination of the data suggests that the $Met^1$ allele is largely responsible for the significance seen.

*Adult survival:* The time to 50% survival was measured for both males and females of each strain (Figure 5). No significant difference either among strains ($F = 2.17$, $P = 0.08$) or between wild type and pooled *Met* ($F = 0.005$, $P = 0.94$) was evident. Therefore, adult survival is not strongly affected in *Met* flies.

Overall, the results suggest that the altered putative JH receptor, which allows *Met* larvae and pupae to survive otherwise toxic doses of methoprene, does not severely impair vital functions.

Another important fitness component is development time. The duration of each stage of development was measured for each strain.

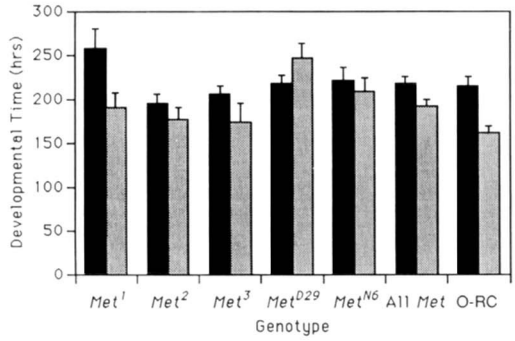


FIGURE 6.—Development time of larvae (■) and (□) pupae of various *Met* and Oregon-RC strains. Each determination is the mean ± SEM number of hours required for 50% pupariation or eclosion in each culture, replicated 8–10 times.

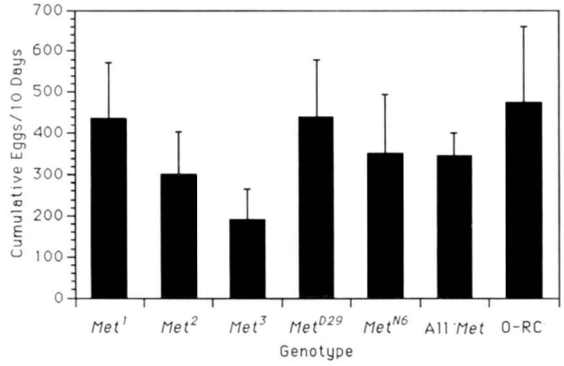


FIGURE 7.—Fecundity of various *Met* and Oregon-RC strains. Each determination is the mean ± SEM cumulative 10-day oviposition for females of the indicted strain, replicated 4–6 times.

*Embryonic development:* Embryonic development in Drosophila requires 20–24 hr at 25°. Since egg hatch was measured 24 hr after oviposition for the egg hatch experiments and no difference was detected between *Met* and wild type, it is clear that the time of embryonic development is not greatly delayed in *Met* flies.

*Larval development:* The length of time from hatching until pupariation was measured (Figure 6). No significant difference between wild type and pooled *Met* ($F = 1.05$, $P = 0.83$) was found. Therefore, larval development time is unaffected by the *Met* mutation.

*Pupal development:* The length of time from pupariation until eclosion was measured (Figure 6). In contrast to larval development, a significant difference between wild type and pooled *Met* ($F = 5.36$, $P = 0.024$) was found. Therefore, pupal development is lengthened in flies homozygous for *Met*.

*Fecundity:* Finally, the fitness component of fecundity was measured for newly eclosed females placed in vials with males of the same strain. Cumulative egg laying for each strain is shown in Figure 7. Females homozygous for some *Met* alleles are less fecund than wild type, and a significant difference was found among strains ($F = 6.70$, $P = 0.001$). However, when pooled *Met* was compared with wild type no significant difference was evident ($F = 1.88$, $P = 0.18$). Therefore, cumulative egg production over a 10-day period is not affected by the *Met* mutant.

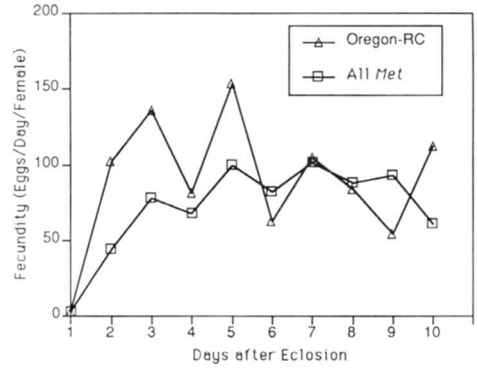


FIGURE 8.—Early fecundity for pooled *Met* and Oregon-RC females. Each point is the mean oviposition rate for females whose cumulative fecundity is described in Figure 7.

Since a role for JH in vitellogenic oocyte development in Drosophila has been clearly shown (POSTLETHWAIT and HANDLER 1979; WILSON 1982), one would expect that *Met* females, having an insensitive putative JH receptor, would have at least a delayed start of vitellogenesis following eclosion. Indeed, $Met^1$ females showed a delayed onset of vitellogenic oocyte development (WILSON and FABIAN 1986). To examine the Met alleles for differences in fecundity early in adult development, the daily fecundity was plotted for the pooled *Met* strains and wild type (Figure 8). Significant differences were found during the first 3 days following eclosion ($F = 5.66$, $P = 0.02$). Therefore, *Met* females have a delay in the onset of egg laying; however, after several days fecundity rises to the wild-type level.

The values obtained for survival and fecundity allowed a calculation of $R$, the replacement rate (FUTUYMA 1986), for the initial 10-day period following eclosion. $R$ is the product of the probability of survival to reproductive age times the mean fecundity (in the present case fecundity during the first 10 days of adult life). This value for wild type was 294 and for pooled *Met* was 191. Therefore, the disadvantage of *Met* flies relative to wild type is evident not only from competition experiments but also in the R value determined from fitness components.

## DISCUSSION

In this study we have addressed several questions that became evident with the discovery of the $Met^1$ mutant: (1) assuming that pest species are likely to have a gene homologous to $Met^+$, could a resistance allele persist in a population following selection with a JH analog, and (2) can we infer more of the role of JH in dipteran insects by an examination of the phenotype of *Met*?

First, there is reason to believe that $Met^+$ homologs may exist in other insects. $Met^+$ affects the binding

affinity of a putative JH receptor and in fact may encode the receptor (SHEMSHEDINI and WILSON 1990). Since JH is a conserved hormone (SCHOOLEY *et al.* 1984), the JH receptor and associated proteins are likely to be conserved as well. Assuming that other insects have a $Met^+$ gene, then mutations of the homolog resulting in reduced JH and JH analog binding affinity seem likely as well.

Second, our results with *Met* suggest that this resistance gene would be rapidly selected in a population under JH analog selection pressure. $Met^1$ flies raised on a dose of methoprene lethal to susceptible flies were found to be both fecund and fertile (WILSON and FABIAN 1987). In addition, $Met^1$ heterozygotes have intermediate resistance (WILSON and FABIAN 1986) and thus a selective advantage. Moreover, the gene is *X*-linked and thus is rapidly expressed in males as its most resistant form in a hemizygote. Finally, methoprene-susceptible flies exposed to sublethal doses of methoprene are reproductively compromised (BOUCHARD and WILSON 1987). Therefore, strong selective forces will favor *Met* individuals in the population.

Although a *Met* allele may rapidly dominate a population under selection pressure, it is clear from the present work that when selection is relaxed, *Met* flies will be rapidly outcompeted by wild type. Both the population cage and the single-generation competition showed wild-type flies to dominate in competition with *Met*. An analysis of the fitness components showed three factors–pupal development time, pupal survival and early fecundity–to be reduced in *Met* flies. Although no drastic reduction in any fitness component was seen, apparently these three summed to severely reduce competition with wild type.

It is possible that the lack of isogenicity among the *Met* strains was responsible for one or more of the differences observed. Some of the alleles originated from a common progenitor strain (Table 1) and therefore have genomic backgrounds that are related. But, no effort was made to achieve isogenicity, for the reason previously given. Had we been interested in assessing the strength of each allele, an isogenetic background would have been critical. Instead, a relatively large number of alleles were studied, and comparisons were made between Oregon-RC and pooled *Met* whenever possible. If one or more of the fitness component reductions seen is due to the background genome instead of *Met*, then the fitness of *Met* is even more similar to wild type than is concluded here.

Has the phenotype of *Met* helped our understanding of the role of JH in these insects? JH functions in adult Drosophila are reasonably well understood (reviewed by BOWNES 1982), but no function for JH in preadult Drosophila or any other dipteran insect has been documented. The absence of major effects of *Met* on preadult development suggests that either JH plays no major role in preadult development or that functional redundancy for JH exists in preadults.

Although the conditional phenotype is strong (WILSON and FABIAN 1986), the nonconditional phenotype is very similar to wild-type. It consists of a delay in the onset of vitellogenic oocyte development (WILSON and FABIAN 1986) as well as the effects on pupal survival, pupal development, and early fecundity seen in the present study. One might expect a mutant whose binding affinity for JH is decreased by an order of magnitude (SHEMSHEDINI and WILSON 1990) to have a more pronounced phenotype, especially regarding fecundity, since JH is known to be involved in vitellogenic oocyte development (POSTLETHWAIT and HANDLER 1979; WILSON 1982). There are several possible explanations for the lack of a pronounced oogenic phenotype: (i) recently, a new JH, JH bisepoxide, has been discovered in *D. melanogaster* (RICHARD *et al.* 1989), and this hormone may be a more important gonadotropic JH in these flies than is JH III; perhaps *Met* controls the receptor specific for JH III but not JH bisepoxide, (ii) although JH may be critical for initiating vitellogenic oocyte development, after ovary development is well underway, another hormone or physiological mechanism can substitute for JH if its regulation is impaired. Thus, we see a delay in the onset of vitellogenic oocyte development, subsequent reduction in early fecundity, but fecundity approaching wild type after several days (Figure 8). Finally, (iii) modifier genes may act to counteract fitness impairment, although we have seen no evidence for this possibility during stock and chromosomal manipulations of *Met* alleles.

This study demonstrates that the fitness assessment of a strain of resistant insects must include competition studies as well as fitness component determinations. Had we measured only the fitness components, we would have concluded from the modest reduction in the three components that, once selected, *Met* flies would remain in high proportion in a population following relaxation of selection. But it is clear from the competition studies that *Met* flies would be rapidly displaced after relaxation. Therefore, either (i) the observed "modest" reduction in several of the components affected competition much more strongly than might have been predicted, or (ii) another fitness component, such as female receptivity or sperm vigor, is drastically altered in *Met* flies and has major responsibility for the competitive disadvantage. Defining the components of fitness is not an easy task. The biology of the insect under question must be well known to understand and measure each of them. Moreover, the importance of one component may differ under competitive *vs.* noncompetitive conditions. Although important fitness components can be evaluated during an examination of survival and fecundity, other less

easily measured components will be overlooked and may impact strongly on fitness. In summary, our work supports the conclusion of others (PROUT 1971; SIMMONS and CROW 1977; ROUSH and MCKENZIE 1987) that total fitness is difficult to assess on the basis of fitness components alone.

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