

The role of adaptation to host plants in the evolution of reproductive isolation: negative evidence from *Tetranychus urticae* Koch

James D. Fry*‡

Department of Biology, University of Michigan, Ann Arbor, MI 48109 USA

(Received 20 December 1996; accepted 10 April 1998)

ABSTRACT

Reproductive isolation between demes of a phytophagous arthropod population that use different host plant species could evolve in two different ways. First, adaptation to different host species might result in reproductive isolation as a pleiotropic by-product. Second, if adaptation to one host species strongly reduces fitness on others, selection could favour mechanisms, such as host fidelity and assortative mating, that restrict gene flow between host-adapted demes. A laboratory selection experiment on the broadly polyphagous spider mite *Tetranychus urticae* gave information on these possibilities. A population allowed to adapt to tomato plants showed increased survival, development rate and fecundity on tomato relative to the base population from which it was derived. In spite of the large difference between the tomato-adapted and base populations in performance on tomato plants, the two populations showed no evidence of reproductive isolation, as measured by the hatching rate of eggs laid by F₁ hybrids between the lines. Furthermore, a genetically variable population formed by hybridizing the tomato-adapted and base populations did not show evidence for a decline in ability to survive on tomato after more than ten generations of mass rearing on lima bean, indicating that tomato-adapted genotypes suffered little or no selective disadvantage on bean. These results give no support for the role of host plants in the evolution of reproductive isolation in *T. urticae*.

Exp Appl Acarol 23: 379–387 © 1999 Kluwer Academic Publishers

Keywords: Host race formation, speciation, spider mites, trade-offs, host plant adaptation, reproductive isolation.

INTRODUCTION

Adaptation of phytophagous arthropod populations to different host species could cause the populations to become reproductively isolated by either of two routes. First, if adaptation to one host is maladaptive with respect to fitness on others, selection could favour host specialization and avoidance of mating with individuals that developed on other hosts (Maynard Smith, 1966; Diehl and Bush, 1989). A second possibility and one that has less often been considered is that genetic

*To whom correspondence should be addressed at: Tel: +1 801 797 2485; Fax: +1 801 797 1575; e-mail: jdfry@biology.usu.edu

‡Present address: Department of Biology, Utah State University, Logan, UT 84322-5305, USA.

changes involved in adaptation to a host might have pleiotropic effects that cause reproductive isolation from ancestral populations as a by-product. This possibility is supported by observations that laboratory artificial selection experiments with *Drosophila* have sometimes resulted in partial reproductive isolation between populations selected in different directions or between selected and unselected populations (reviewed in Rice and Hostert, 1993).

The two-spotted spider mite, *Tetranychus urticae* Koch, is a leaf-feeding mite that has been recorded from hundreds of different plant species in many different families (Jeppson *et al.*, 1975). Different isolates of *T. urticae*, even those collected only a few kilometres apart, typically show varying degrees of reproductive incompatibility with each other (reviewed in De Boer, 1985). The type of reproductive incompatibility that has been reported most often is low hatch rates of eggs laid by F_1 females, particularly the unfertilized (male) eggs in this haplodiploid species. Other types of incompatibility, such as low fertilization rates and hybrid inviability, have also been reported. Little is known about the events that lead to the development of these reproductive incompatibilities. The purpose of this note is to consider whether adaptation to different host plants might play a role in their origin.

The work reported here is based on a set of laboratory populations described by Fry (1988, 1989, 1990). One population (the 'tomato line') was allowed to adapt to a tomato variety on which its survival and fecundity was initially low. Two other populations (the 'bean' and 'minimum selection' lines), derived from the same base population as the tomato line, were kept on lima bean, a host on which survival and fecundity was initially high. These populations served as controls for evolutionary changes in the tomato line (see Fry (1989) for details). The tomato line rapidly evolved higher ability to survive on tomato than the bean and minimum selection lines, which showed no consistent change in their ability to survive on tomato (Fig. 1). Mites from the tomato line also evolved faster development and higher fecundity on tomato than the control lines (Fry, 1989) and also showed higher acceptance of tomato than the control lines (Fry, 1989).

The results of two experiments involving hybridizing the tomato and bean lines are reported here. These experiments shed light on the possible role of adaptation to different hosts in the origin of reproductive incompatibilities in *T. urticae*, by either of the two mechanisms described above.

EXPERIMENTS

Experiment 1: Hatch rate of eggs laid by F_1 hybrids between the tomato and bean lines

To test the hypothesis that adaptation to different hosts can cause the evolution of reproductive isolation as a pleiotropic by-product, the hatch rate of eggs laid by females formed by hybridizing the tomato and bean lines was examined.

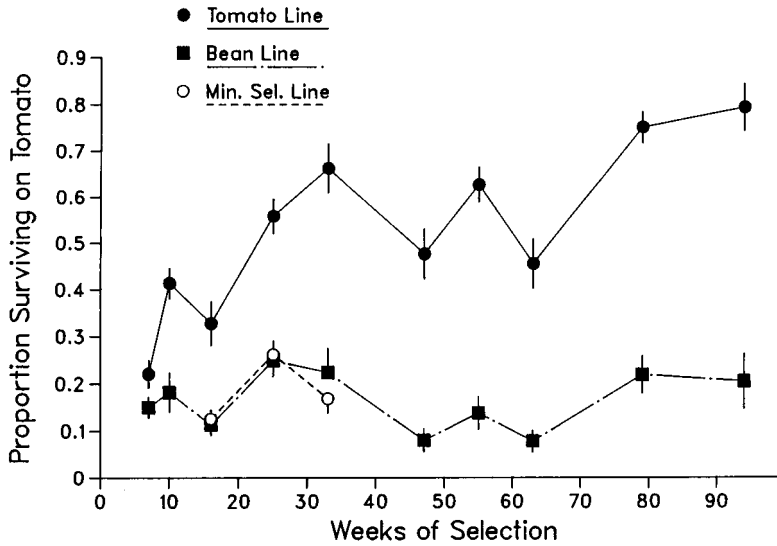


Fig. 1. Proportion of progeny of the tomato, bean and minimum selection lines of *T. urticae* surviving in tanglefoot enclosures on leaflets of potted tomato plants. Each point is the mean from 10–21 (usually 17–21) enclosures, with a mean of 22 progeny per enclosure. Bars represent one standard error above and below the mean. Results taken from Fry (1988, 1989, 1990).

Experiment 2: Fitness trade-offs between tomato and lima bean

Two ‘relaxed selection’ lines derived from the tomato line and maintained on lima bean declined in their ability to survive on tomato after 10–15 generations (Fry, 1990). This was interpreted as evidence that tomato-adapted genotypes were at a selective disadvantage on bean (Fry, 1990); if true, this would lend support to the notion that ‘trade-offs’ between fitness on different hosts exist. In theory, such trade-offs could cause selection to favour mechanisms that reduce the probability of mating between *T. urticae* demes adapted to different hosts (Maynard Smith, 1966; Diehl and Bush, 1989). The survival declines on tomato in the RS lines were relatively slight, however, and took place slowly. This could mean that selection against tomato-adapted genotypes on bean was relatively weak or that the survival declines were not caused by selection but by some other factor (e.g. genetic drift). Alternatively, genetic variation in the tomato line may have been mostly exhausted by the time the RS lines were established. If the latter explanation is correct, then a line created by hybridizing the tomato and bean lines should show greater survival declines on tomato in response to being maintained on bean. The results of an experiment with such a ‘hybrid RS’ line are reported here.

MATERIALS AND METHODS

The establishment and maintenance of the tomato, bean and minimum selection lines, growing conditions for the plants and other experimental details are reported in Fry (1988, 1989, 1990).

Crosses between the tomato and bean lines were conducted 55 weeks after the tomato line was established. Crosses were performed by placing a single quiescent deutonymph female together with two males from the opposite line on a bean leaf disc. Twenty such crosses were conducted in each direction. After they emerged and mated, the females were moved to a fresh disc and allowed to lay eggs (approximately ten) for 1 day. One quiescent deutonymph F_1 female was taken from each of the resulting broods and moved to a fresh disc, where it was allowed to emerge and lay eggs. No males were provided, so all the eggs laid by the F_1 females were unfertilized and therefore gave rise to haploid males. The females were removed after laying approximately 40 eggs, the eggs counted and the number hatching over the next few days recorded. Control (intraline) crosses were not conducted at this time, but data on the hatch rate of eggs laid on bean leaf discs by 50 females from each of the tomato and bean lines, collected after 33 weeks of selection in the tomato line, were available for comparison. These females had been mated to males from their own lines.

To create the hybrid RS line, ten sib-mated F_1 females from each of four of the above crosses in each direction (total 80 females) were combined with 15 males from each line on detached lima bean leaves in a plastic shirt box (see Fry (1989) for rearing methods). The population was allowed to expand to well over 1000 mites and was maintained for 10 months. Generations were overlapping, but mean generation time was probably no more than 3 weeks (J.P. Fry, unpublished observations). Seven months after the hybrid RS line was created, a sample of mites from this line was compared to mites from the tomato and bean lines for survival on leaves of potted tomato plants. These tests were performed by allowing mated females from each line to lay eggs inside tanglefoot enclosures on the upper surfaces of the leaves ($n = 6$ leaves, with one enclosure per line on each leaf) and examining the survival of the progeny (mean 20 per enclosure; see Fry (1989) for details). Three months later, a second survival comparison on tomato was performed, this time between male offspring from the hybrid RS line and male offspring of newly created F_1 hybrids between the bean and tomato lines ($n = 7$ leaves); the F_1 hybrids consisted of approximately equal numbers of females from each of the two reciprocal crosses. All male broods were obtained by using unmated females to lay eggs.

RESULTS

Approximately 93% of the eggs laid by F_1 females hatched (Table 1). Although the hatch rate of eggs laid by pure line females was slightly higher, such a slight

TABLE 1

Hatching succes of eggs laid by *T. urticae* females on lima bean leaf discs

Females	Number of eggs	% inviable
Bean line	389	4.4
F ₁ (bean line females × tomato line males)	697	6.5
F ₁ (tomato line females × bean line males)	564	7.6
Tomato line	412	4.4

difference is expected, because the pure line females were fertilized while the hybrid females were not. Hatch rates of eggs laid by mated females in *T. urticae* are typically one to three percentage points higher than those of unmated females (Overmeer and van Zon, 1976). Therefore the results in Table 1 give no evidence that adaptation to tomato in the tomato line caused the evolution of reproductive isolation from the bean line.

Seven months after the hybrid RS line was established, mean proportions surviving (\pm SE) on tomato of bean line, hybrid RS line and tomato line mites were 0.23 (\pm 0.07), 0.62 (\pm 0.06) and 0.85 (\pm 0.04), respectively (Fig. 2). Analysis of variance on the angular-transformed proportions revealed that the three lines differed significantly ($p = 0.0002$), but a linear contrast (Larsen and Marx, 1981) between the hybrid RS line and the mean of the tomato and bean lines was not significant ($p = 0.35$). The hybrid RS line started with half of its genes from each of the tomato and bean lines; therefore if tomato-adapted genotypes had been strongly selected against on bean, the hybrid RS line should have had lower survival than the mean of the tomato and bean lines, assuming that the genes

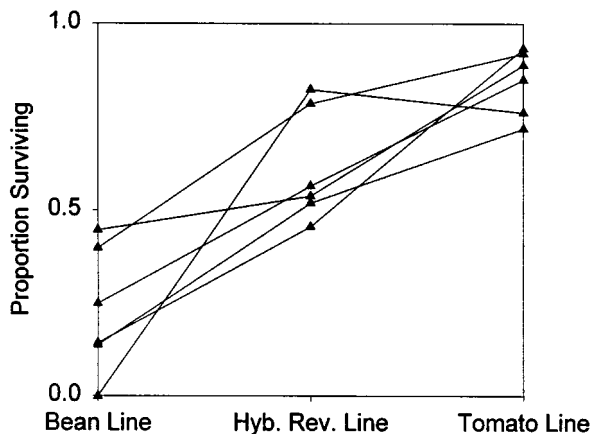


Fig. 2. Proportion surviving on tomato of cohorts of the bean line, hybrid reversion line, and tomato line of *T. urticae*, 7 months after the hybrid reversion line was established. Each line in the figure gives the results from separate tanglefoot enclosures on a single tomato leaf.

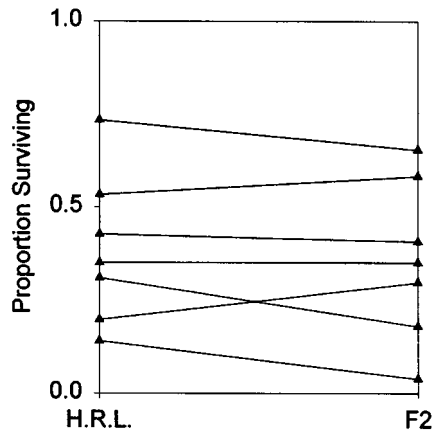


Fig. 3. Proportion surviving on tomato of all-male broods of the hybrid reversion line (HRL), and of male progeny of F_1 hybrids between the bean and tomato lines (F2) of *T. urticae*, 10 months after the hybrid reversion line was established. Each line in the figure gives the results from a separate tanglefoot enclosure on a single tomato leaf.

causing the survival differences had approximately additive effects (i.e. no dominance or epistasis).

Ten months after the hybrid RS line was established, the survival on tomato of males from the line was compared to that of male progeny of F_1 hybrids between the tomato and bean lines (the latter progeny have half their genes derived from each parent line). The mean proportions surviving (\pm SE) of the two groups were $0.39 (\pm 0.08)$ and $0.36 (\pm 0.08)$, respectively (Fig. 3). Analysis of variance on the angular-transformed proportions revealed no significant difference ($p = 0.37$). By the same logic as above, if tomato-adapted genotypes had been strongly selected against on bean, the hybrid RS line males should have exhibited lower survival in this comparison.

DISCUSSION

The results reported here do not support the view that adaptation of *T. urticae* populations to different hosts is likely to lead to the evolution of reproductive isolation between them. Although the tomato and bean lines differed strongly in their survival on tomato (Figs 1 and 2), females formed by hybridizing the lines laid eggs that hatched at a normal rate. Furthermore, a population started with 50% of its genes from each line and maintained on lima bean for over ten generations did not show evidence of a decline in ability to survive on tomato, as would be expected if tomato-adapted genotypes had been selected against on bean.

The prediction that eggs laid by hybrids between the tomato and bean lines might hatch at a reduced rate could seem strange, but results of Overmeer (1966) lend

support to the prediction. Overmeer (1966) selected two populations of *T. urticae*, derived from the same base population, for resistance to the acaricide Tedion. Hybrids between each of the selected populations and the unselected base population laid a high percentage of inviable eggs, but this intersterility was not observed in crosses between the two selected populations. These results indicate that the intersterility evolved as a direct consequence of acaricide selection and suggest that selection for ability to survive on a toxic host plant could have the same effect. It seems unlikely, however, that acaricide selection is the only cause of the evolution of partial reproductive incompatibility between demes or populations of *T. urticae*, because incompatibility is often observed between samples collected from non-agricultural areas (De Boer, 1980).

The failure of the hybrid RS line to show evidence of a decline in ability to survive on tomato raises the question of what caused the declines in survival on tomato of the two RS lines established from the tomato line described by Fry (1990). Although it was argued earlier that the declines gave evidence for selection against tomato-adapted genotypes on lima bean (Fry, 1990), this hypothesis is weakened by the results with the hybrid RS line reported here. Furthermore, the tomato line did not show reduced survival or fecundity on lima bean compared to the bean line, although a slight difference could not be ruled out (Fry, 1990). Perhaps the most parsimonious explanation for the declines in survival on tomato of the two RS lines is genetic drift or some other process not involving selection. In a selection line at or near a selection limit, as the tomato line apparently was when the two RS lines were established (Fry, 1990), genetic drift could cause a predictable decline in the previously selected character upon relaxation of selection. For example, suppose that part of the selection response of the tomato line was caused by a quantitative character under strong stabilizing selection on tomato but neutral on bean. At the selection limit on tomato, the tomato line's mean for the character should be close to the optimum. In an RS line transferred to bean, genetic drift of the trait mean away from the optimum in either direction would reduce mean fitness on tomato. In contrast, genetic drift would not predictably cause a reduction in fitness on tomato in a hybrid RS line like the one described here, because such a line is not initially at a fitness optimum on tomato, so drift could either decrease or increase its fitness on tomato.

It is also possible that recurrent mutations that reduced fitness on tomato but were nearly neutral on bean contributed to the survival declines on tomato of the RS lines. Kawecki (1994) and Fry (1996) discussed the possible theoretical consequences of deleterious mutations with host-specific effects in phytophagous arthropods and Fry *et al.* (1996) presented evidence that many deleterious mutations in *Drosophila* act in an environment-specific manner.

All of the above possibilities are speculative, but serve to illustrate that it is not necessary to invoke selection to explain the survival declines on tomato of the RS lines (Fry, 1990). Furthermore, regardless of the explanation for those declines, the totality of the evidence (Fry, 1990; this study) indicates that tomato-adapted genotypes were not at a strong fitness disadvantage on bean. This means that

selection taking place in a *T. urticae* population inhabiting lima bean would be unlikely to favour avoidance of mating with mites immigrating from tomato. There could still be selection in a population on tomato against mating with mites immigrating from other hosts, as those mites would be expected to be less well adapted to tomato than the resident mites. Nonetheless, the evolution of reproductive isolation along host plant lines is expected to take place more readily in the presence of trade-offs than in their absence, all other things being equal (Kawecki, 1996).

The results reported here parallel some results of previous workers. De Boer (1979) found no evidence for reproductive isolation, as measured by hatching rates of eggs laid by hybrid females, between *T. urticae* colonies maintained on different host species for approximately 9 months. A cucumber-adapted selection line created by Gould (1979) did not show reduced survival on three other host species as a consequence of adaptation to cucumber, although two RS lines returned to lima bean did show some evidence for a decline in ability to survive on cucumber. Current evidence therefore does not support the hypothesis that adaptation to different hosts is likely to lead to the evolution of reproductive isolation between *T. urticae* populations. This conclusion must be taken as very preliminary, however, for two reasons. First, evidence for possible trade-offs has been sought in only a few *T. urticae* populations and the experiments involved only a few host species. Second, adaptation to different hosts could cause reproductive isolation as a pleiotropic by-product by mechanisms other than reduced hatch rate of eggs laid by F₁ females, such as behavioural barriers to mating (Rice and Hostert, 1993). Further work is therefore needed to evaluate the role of host plants in the evolution of reproductive isolation in *T. urticae*, as well as in other phytophagous arthropods.

ACKNOWLEDGEMENTS

This manuscript was presented at the Third Symposium of the European Association of Acarologists (EURACC) which was held on 1–5 July 1996 in Amsterdam, The Netherlands.

REFERENCES

- De Boer, R. 1979. Investigations concerning the development of reproductive incompatibilities between populations of the spider mite, *Tetranychus urticae*. *Zeitschrift Angew. Entomol.* 87: 113–121.
- De Boer, R. 1980. Genetic affinities between spider mite *Tetranychus urticae* (Acarina: Tetranychidae) populations in a non-agricultural area. *Entomol. Exp. Appl.* 28: 22–28.
- De Boer, R. 1985. Reproductive barriers. In *Spider mites: their biology, natural enemies and control*, Vol. 1A, W. Helle and M.W. Sabelis (eds), pp. 193–199. Elsevier, Amsterdam.

- Diehl, S.R. and Bush, G.L. 1989. The role of habitat preference in adaptation and speciation. In *Speciation and its consequences*, D. Otte and J. Endler (eds), pp. 345–365. Sinauer, Sunderland, MA.
- Fry, J.D. 1988. Genetic variation and genetic correlations in host-specific fitness in the phytophagous mite *Tetranychus urticae* Koch. PhD dissertation, University of Michigan, Ann Arbor, MI.
- Fry, J.D. 1989. Evolutionary adaptation to host plants in a laboratory population of the phytophagous mite *Tetranychus urticae* Koch. *Oecologia* 81: 559–565.
- Fry, J.D. 1990. Trade-offs in fitness on different hosts: evidence from a selection experiment with a phytophagous mite. *Am. Nat.* 136: 569–580.
- Fry, J.D. 1996. The evolution of host specialization: are ‘trade-offs’ overrated? *Am. Nat.* 148: S84–S107.
- Fry, J.D., Heinsohn, S.L. and Mackay, T.F.C. 1996. The contribution of new mutations to genotype-environment interaction for fitness in *Drosophila melanogaster*. *Evolution* 50: 2316–2327.
- Gould, F. 1979. Rapid host range evolution in a population of the phytophagous mite *Tetranychus urticae* Koch. *Evolution* 33: 791–802.
- Jeppson, L.R., Keifer, H.H. and Baker, E.W. 1975. *Mites Injurious to Economic Plants*. University of California Press, Berkeley, CA.
- Kawecki, T.J. 1994. Accumulation of deleterious mutations and the evolutionary cost of being a generalist. *Am. Nat.* 144: 833–838.
- Kawecki, T.J. 1996. Sympatric speciation driven by beneficial mutations. *Proc. R. Soc. London B* 263: 1515–1520.
- Larsen, R.J. and Marx, M.L. 1981. *An Introduction to Mathematical Statistics and its Applications*. Prentice-Hall, Englewood Cliffs, NJ.
- Maynard Smith, J. 1966. Sympatric speciation. *Am. Nat.* 100: 637–650.
- Overmeer, W.P.J. 1966. Intersterility as a consequence of insecticide selections in *Tetranychus urticae* Koch (Acari: Tetranychidae). *Nature* 209: 321.
- Overmeer, W.P.J. and van Zon, A.Q. 1976. Partial reproductive incompatibility between populations of spider mites (Acarina: Tetranychidae). *Entomol. Exp. Appl.* 20: 225–236.
- Rice, W.R. and Hostert, E.E. 1993. Laboratory experiments on speciation: what have we learned in 40 years? *Evolution* 47: 1637–1653.