Host acceptance and life-history traits in *Drosophila busckii*: tests of the hierarchy-threshold model

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The hierarchy-threshold model for host-choice in insects predicts positive genetic correlations between use of different hosts, and between host use and the number of eggs carried by females. Life history theory predicts negative covariance between reproductive capacity and lifespan; hence host use and lifespan should be negatively correlated. These predictions were tested in half-sib breeding designs with the cosmopolitan *Drosophila busckii*. The predictions of the hierarchy-threshold model were met, supporting previous findings with other *Drosophila*. Predictions from life-history theory were not met for the population studied.

INTRODUCTION

A number of studies have recently documented the presence of genetic variance affecting host choice behaviour within natural populations of plant-feeding insects (Tavormina, 1982; Jaenike and Grimaldi, 1983; Via, 1984; Lofdahl, 1987; Jaenike, 1987; Thompson, 1988*a*; Courtney and Chen, 1988). In reviewing these and other data, Futuyma and Petersen (1985) have drawn attention to the scarcity of information regarding the factors that maintain such variation within populations. In particular, Futuyma and Petersen point out the need for studies of genetic covariance of other characters, and of the nature of genetic correlations between characters. Genetic correlations due to pleiotropy may impose constraints on the evolution of host choice behaviour. Similarly, correlated responses to natural selection may explain both observed patterns of host use, and the maintenance of additive variance for host selection. Here we test a model which predicts pleiotropic interactions between host use characters, using a population of the cosmopolitan *Drosophila busckii*.

Courtney *et al.* (1989) have proposed a comprehensive theory for the mechanistic control of host choice behaviour. The “hierarchy-threshold” model which they advance makes a number of predictions concerning genetic variance for host use, and covariance with other characters. The model shows that pleiotropy between traits is

expected to maintain genetic variability. Courtney *et al.* argue that insects rank potential hosts in order of decreasing acceptability; high ranking hosts are always acceptable, while low-ranking hosts may become acceptable, dependent upon environmental conditions. An insect accepting a low-ranking host must therefore also accept all higher-ranked hosts. The model finds some empirical support in studies by other workers, notably Wiklund (1981, 1982), Singer (1982, 1986), Lofdahl (1987) and Thompson (1988*a*). Factors influencing the rate at which hosts become accepted include current eggload borne by females (Fitt, 1986) and adult experience (Jaenike, 1982, 1983). Specific predictions made by Courtney *et al.* (1989) include: (1) Significant levels of additive genetic variance for use of low-ranking hosts will be maintained in populations, since such variance is rarely subject to direct selection (this holds true even if the host is *never* experienced in the population in question, *i.e.*, it is a novel host); (2) less variance is expected for use of higher-ranked hosts (unless they are novel) due to direct selection on such traits; (3) significant positive genetic correlations are expected between use of different low-ranking, novel hosts; (4) there will be genetic correlations between use of low-ranking hosts and factors influencing acceptance (high eggload increases acceptance—therefore we expect a positive genetic correlation between these traits); (5) no such correlations are expected for characters

measured against use of high-ranking hosts. These predictions have been met by several studies. Lofdahl (1987) reports additive genetic variance for use of a novel cactus species by *D. mojavensis*; similar results obtain for *D. suboccidentalis*, a mushroom feeder, when presented with two novel hosts, commercial mushroom (*Agaricus campestris*) and cucumber (*Cucumis sativa*) (Courtney and Chen, 1988; Chen, 1987). Courtney *et al.* (1989) report a significant positive genetic correlation between use of low-ranked *Cucumis* and eggload in 6-day-old *D. suboccidentalis*, but no correlation between use of higher-ranked *Agaricus* and eggload.

D. suboccidentalis might be seen as an atypical species. It is an ecologically monophagous species (*sensu* Smiley, 1978), which uses only fungi of genus *Ramaria* in the study population of the Cascade mountains in western Oregon (Courtney *et al.*, 1989). Here, other fungi are rare and unpredictable, and *Ramaria* is the only relatively abundant resource. Monophagous populations are, by definition, subject to little direct selection on host choice. Results for *D. suboccidentalis* might therefore reflect the special circumstances of a monophagous species. The present study was carried out to determine whether similar results may be seen in polyphagous species, which live under more variable selection regimes. The cosmopolitan *D. busckii* was chosen because of its extreme breadth of diet in the wild (many rotting substrates, including mushrooms, are used), and because it is distantly related to *D. suboccidentalis*. *D. busckii* is in subgenus *Drosophila*; *D. suboccidentalis* is a member of the *quinaria* complex of subgenus *Dorsilopha*. If the predictions of the hierarchy-threshold model are met for such ecologically and taxonomically diverse species, the theory may be robust.

An additional interest was to incorporate into our experimental design consideration of another life-history character, adult lifespan. Considerable attention has been given to theories of life-history evolution which rely on antagonistic pleiotropy between life-history components. For instance, Rose and Charlesworth (1981) show that, in some populations of *D. melanogaster*, early fecundity and lifespan are negatively genetically correlated. Some other studies support this result (*e.g.*, Roach, 1986; Luckinbill *et al.*, 1987; Scheiner *et al.*, 1989) but others find equivocal (Hughes and Clark, 1988) or even opposite results (Giesel *et al.*, 1982). Whatever the generality of the results, antagonistic pleiotropy between fecundity and lifespan could have important ramifications given that fecundity itself is predicted to covary with host use. A deriva-

tive prediction of life-history theory and the hierarchy-threshold model is thus that we expect *negative* covariance between lifespan and acceptance of low-ranking hosts, due to pleiotropic effects through reproductive allocation.

METHODS

We carried out a standard half-sib breeding design using tenth generation descendants of *D. busckii* caught at Eugene, Oregon on mushroom baits. Stocks were maintained at high population numbers (>1000) over Carolina instant *Drosophila* medium at 25°C, and under constant light. In all, 23 males were successfully mated to an average of 5.5 females, producing 126 full-sib families. Families were raised on instant medium as before. Half-sib families were distributed randomly among vials and with respect to position to the rearing chamber and time of onset. This procedure ensures that common environment (larval rearing media) effects did not influence the estimates of heritability or genetic correlations, derived from half-sib designs (Falconer, 1981). Flies were sexed at emergence, and the sexes separated. Females were then divided among four treatments. One group was dissected at age 6 days, to determine the eggload carried. Eggload is measured as an index of reproductive allocation, independent of mating history. Eggload was measured in virgins, because mated females would begin to lay eggs. This seems a reasonable way to study host choice, since females mate at the oviposition site, and must therefore sometimes approach hosts as virgins. A second group was maintained (virgin) over medium, and the lifespan of each individual recorded. The remaining flies were mated at day 6 and exposed for 24 hours to approximately 1 g of fresh material of one of two hosts: *A. campestris* or *C. sativa*. Oviposition substrates were then examined for the presence of eggs; if one egg or more was found, then the host was scored as accepted. The total number of eggs laid on each host was also recorded. Only one host was offered to each fly; This procedure is necessary because exposure to a host changes the behaviour of females on other hosts (Courtney and Gardner unpublished) and may alter both fecundity and survivorship.

Heritabilities of the numbers of eggs laid on either host, of female lifespan and of eggload were estimated using standard methods employing nested ANOVA (Falconer, 1981). Heritabilities of host acceptance were calculated using methods applicable to threshold traits (Robertson and Lerner, 1949); standard errors for unbalanced designs are not calculable, and significance of these

threshold characters were estimated from χ^2 analysis. Genetic correlations were estimated from correlations across family means, following the suggestions of Via (1984) for situations where only one character can be measured per individual. This is a conservative procedure which, relative to other measures of r_A , tends to underestimate correlations of characters; it does have the advantage of employing standard tests of significance. Percentages were arcsine square-root transformed before analysis.

RESULTS

Heritabilities of the characters studied are shown in table 1. As predicted, acceptance of the low-ranking host, cucumber, showed significant additive genetic variance, even though this host must be very rarely encountered and used in the field. Heritability of acceptance of the high-ranking host, *A. campestris*, was not significantly different from zero, again as predicted from the hierarchy-threshold model. Heritabilities of the numbers of eggs laid on either host and of life-history characteristics were low, and only that of eggload was significantly different from zero.

A significant positive genetic correlation was found between acceptance of the two hosts (table 2). Acceptance of the low-ranking host was also positively correlated with eggload. The num-

bers of eggs laid on the two hosts were not however correlated, although each did weakly correlate with eggload. We interpret these results as supporting the hierarchy-threshold model. Acceptance (whether or not at least one egg is laid) is not closely associated with the numbers of eggs laid by accepting females. This argues that acceptance, and the numbers of eggs laid following acceptance are largely independent characters in *D. busckii*. Acceptance of the low-ranking host is positively correlated with eggload as predicted. Use of the high-ranking host is not correlated with eggload, again as predicted. Correlations between eggload and the number of eggs laid on either host probably reflect rather simple effects of the number of eggs available for laying.

Predictions based on expectations of antagonistic pleiotropy between lifespan and eggload were not met (table 2). No significant relationship between these two characters was found. Neither did acceptance of cucumber correlate negatively with lifespan. Eggload may not be the most appropriate measure of reproductive allocation; nevertheless, significant additive genetic variance was detected for this trait, indicating the possibility of detecting significant correlations. An unexpected result was the significant *positive* correlation between lifespan and acceptance of the high-ranking host. Possible interpretations include: (1) the widespread presence of deleterious mutations in inbred lines, leading to positive correlations between life-history components (Rose, 1984). We reject this interpretation because our stocks were recently derived from natural populations, and were kept at high numbers. Also, no correlation between lifespan and eggload was seen, as predicted from this explanation. (2) Interaction between behaviours favouring survival and those promoting acceptance of a normal host. This might for instance occur where adults are attracted to feed on the oviposition substrate. We do not favour this hypothesis, and currently lack a satisfactory explanation for the observed correlation.

Table 1 Heritabilities of traits in the *D. busckii* population

	h^2	S.E.	χ^2	P
E. Eggload	0.24	0.11	—	—
L. Longevity	0.16	0.18	—	—
Ac Acceptance of <i>Cucumis</i>	0.25		42.5	0.01
Aa Acceptance of <i>Agaricus</i>	0.08		29.3	ns

Acceptance traits were treated as threshold characters, for which no standard errors are calculable for unbalanced data sets; significance levels are determined by χ^2 statistic for these traits.

Table 2 Genetic correlations between traits, as described in the text. $n = 23$ in all cases

	L.	Ac	Aa	Nc	Na
E. Eggload	-0.038	0.628***	0.234	0.466*	0.437*
L. Longevity		-0.036	0.527***	-0.132	0.330
Ac Acceptance of <i>Cucumis</i>			0.552**	0.125	
Aa Acceptance of <i>Agaricus</i>					0.290
Nc Number of eggs on <i>Cucumis</i>					0.151
Na Number of eggs on <i>Agaricus</i>					

Significance levels $P < 0.05^*$, 0.01^{**} , 0.005^{***} . Full-sib correlations did not yield additional information, except that the number of eggs laid by sibs on the two hosts were significantly correlated ($r = 0.452$, $P < 0.01$)

DISCUSSION

The predictions of the hierarchy-threshold model (Courtney *et al.*, 1989) have been met for *D. busckii*. Acceptance of hosts does appear to depend on physiological status of the female. Observed patterns of genetic correlations imply pleiotropy, since it is unlikely that selection in the uniform lab environment would maintain sufficient levels of linkage disequilibrium to explain such high genetic correlations. All observations therefore support the proposition of Courtney *et al.*, that host choice is strongly affected by internal factors, and that pleiotropy between characters may underlie and help to explain the observed high levels of genetic variance for host acceptance behaviour.

Our results for *D. busckii* confirm earlier, similar findings in *D. suboccidentalis*. These two species are at opposite ends of the resource-breadth continuum, since *D. suboccidentalis* utilises a narrow range of hosts (so far we have found it only on *Ramaria*) while *D. busckii* uses many substrates including mushrooms, skunk cabbage, rotting wood and garbage. These two species are also taxonomically distant, occurring in different subgenera of *Drosophila*. That the underlying genetic structure of the two species is so similar argues that the hierarchy-threshold model is not sensitive to taxonomic and ecological considerations, and may be generally applicable.

The exact values for h^2 and r_A in any lab study must always be treated with caution. Nevertheless our results are in broad agreement with earlier results for *D. mojavensis* and *D. suboccidentalis* where heritability of acceptance of novel hosts was low even if significant (12–22 per cent) (Lofdahl, 1987; Courtney and Chen, 1988; Courtney *et al.*, 1989). Correlations between characters were relatively high compared to other behavioural studies (Roff and Mousseau, 1987) but comparable to our earlier results for *D. suboccidentalis*. This again supports our mechanistic explanation that correlations result from the constraint implied by physiological processes affecting both egg maturation and host choice.

An additional life-history component, female longevity, was not correlated with host choice in the manner predicted. This suggests that selection could operate on variation affecting longevity independently from selection acting on variation affecting host acceptance. Nevertheless these characters may show some association. Long-lived flies will tend to have more opportunities for acceptance or rejection of hosts. The hierarchy-threshold model also suggests that animals accept

lower-ranking hosts following host deprivation (e.g., after periods of inclement weather, when dispersal is prevented, or when host abundance is low). Longer-lived flies may well then be the part of the population most likely to survive inter-host periods, and hence to be subject to selection for acceptance of lower-ranked hosts. *A priori* then, we may expect *positive* genetic correlations between longevity and acceptance of low-ranked hosts, due to linkage disequilibrium. We should not however detect this pattern (expected under field conditions) in our lab-reared populations unless linkage is very strong.

Our results indicate the presence within populations of *D. busckii* of genetic variance for use of a novel host *Cucumis*. If ecological conditions ever arose where use of this host was favoured, this genetic variance would already be present in the population, and available for the operation of selection. As pointed out by Lofdahl (1987) and Courtney *et al.* (1989), the presence of genetic variance for use of novel hosts implies that host shifts may occur rapidly. What maintains this variance? Our results clearly implicate correlated selection on reproductive allocation as one plausible explanation. If eggload early in life is subject to fluctuating selection, we may expect genetic variance for this character to be maintained. Given the variable thermal environment and patchy host distribution of both *D. busckii* and *D. suboccidentalis*, we feel that further attention should be given to effects on female eggload.

Our results also suggest that selection on host use may result in responses in other characters. In particular, selection against use of suboptimal hosts may initially result in lowered early fecundity. However we believe that such selection against a fitness component would result in a change in the correlation between the traits; in this case the internal threshold for host acceptance would rise, and the correlation would weaken. Selection on acceptance of one host will also have correlated responses on acceptance of other hosts. If our population of *D. busckii* had experienced strong directional selection for use of *Cucumis* we would also expect the population to readily accept *Agaricus* and other hosts ranked higher than *Cucumis*. This effect has potential importance in insect pests, where colonists onto one novel host (crop) are also likely to be colonists of other hosts.

Our studies have not addressed the relationship of adult and larval characters. In particular we have not considered here the suitability of different hosts for larval development. Both *Cucumis* and *Agaricus* are well able to support larval growth of

D. busckii. Generally, adult oviposition range of insects is less broad than larval dietary tolerance (Wiklund, 1975). We agree with Futuyma (1983), Jermy (1984) and others that understanding oviposition choice is therefore critical to our knowledge of the evolution of host use. The hierarchy-threshold model, which concerns the mechanism of oviposition choice, implies that hosts are ranked in order of acceptability. The evolutionary relationship between acceptability and suitability for larval development is largely unexplored (Thompson, 1988b, but see Rausher, 1984; Rausher and Englander, 1988). Given that selection against use of low-ranking hosts will usually be both weak and sporadic, the exact relationship of adult preference and performance requires much further investigation.

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