Functional Ecology 1993
7, 528–534

Trade-offs with resistance to a granulosis virus in the Indian meal moth, examined by a laboratory evolution experiment

M. BOOTS and M. BEGON
Population Biology Research Group, Department of Environmental and Evolutionary Biology, The University of Liverpool, PO Box 147, Liverpool L69 3BX, UK

Summary

1. When microbial agents are used as pest-control agents, resistance in the host may be selected for. If resistance occurs there are potentially fitness costs due to trade-offs between resistance and other life-history traits. Genotypic trade-offs with resistance to a virus in a lepidopteran host are examined by a micro-evolutionary selection experiment.
2. Six populations of the Indian meal moth, *Plodia interpunctella*, were established, three of which supported a granulosis virus infection (selected insects) while the remaining three acted as virus-free controls.
3. After a period of 2 years, bioassays with the virus showed that selected moths were 1·96-fold more resistant to infection (LD_{50}s) than those derived from the virus-free control populations.
4. Correlated with this increase in resistance were a lengthening of development time, a reduction in egg viability and an increase in pupal weight.
5. These changes in life-history traits suggest that a cost in fitness of 15% (*sensu* Sibly & Calow 1986) is associated with the evolution of the resistance.
6. The importance of fitness costs associated with the development of resistance to pathogens is discussed.

Key-words: Fitness costs, genotypic correlations, *Plodia interpunctella*, resistance to pathogens
Functional Ecology (1993) **7**, 528–534

Introduction

The response to selection acting on one character may be constrained by genetic correlations with other characters (Falconer 1989; Maynard Smith *et al.* 1985). The major cause of such genetic correlations is pleiotropy (Williams 1957), which occurs when a gene has an effect on two or more traits. Should the pleiotropic effects on fitness be antagonistic, the expected result is a negative correlation between the traits or a trade-off. Studies of trade-offs have concentrated on correlations among quantitative life-history traits such as fecundity, development time and reproductive success (Bell & Koufopanou 1986; Stearns 1989). There are, however, many other traits that may affect fitness, one of which is relative resistance to pathogens. Given that individuals differ in this resistance, and that it is likely that this variation is in part genetically determined, it is possible that trade-offs exist between it and other life-history traits. Such trade-offs are the subject of this study. Specifically, we examine the correlations between life-history traits and the development of resistance to a granulosis virus in the Indian meal moth, *Plodia interpunctella* (Hubner). The granulosis viruses are one of the subgroups of the baculoviruses, with infection of the larval host typically occurring when virus occlusion bodies are ingested and then dissolved in the midgut. Viral DNA enters the cytoplasm of the host midgut epithelial cells within a nucleocaspid and is then released at the nuclear membrane. Proliferation of the virus then occurs, resulting in large-scale cell lysis and tissue destruction leading to the death of the host. Subsequently the hypodermis ruptures, releasing large numbers of occlusion bodies into the environment where they become available for ingestion by other hosts (for further details see Granados 1980).

Genotypic trade-offs between pathogen resistance and other fitness traits are important because their extent gives an indication first of the rate at which resistance is likely to evolve and second of the level of

resistance which can be obtained. Furthermore, the cost of resistance will determine the rate at which any evolved resistance would be lost in the absence of the pathogen. An understanding of each of these processes is vital to the understanding of the dynamics of the evolution of pathogen resistance, which is itself essential if pathogens are to be used in biological control programs.

Most studies of the development of resistance to pathogens have not examined any correlated changes in other life-history characters (for example, Harvey & Howell 1965; Watanabe 1967; Briese & Mende 1983). However, in a study of resistance to a virus in *Escherichia coli B* (Lenski 1988) all of the 20 resistant mutants studied showed maladaptive pleiotropic effects, with substantial fitness variations between them. Furthermore, in a comparative study of different strains of *P. interpunctella*, Vail & Tebbets (1990) found that the strains with significantly higher resistance levels to the granulosis virus also had significantly longer development times. It is, however, difficult to infer genetic constraints from the examination of phenotypic correlations (Charlesworth 1980; Stearns 1980; Rose & Charlesworth 1981a, b).

Genetic correlations are concerned with the potential for correlated responses in traits to selection of other traits. As such they are examined directly by selection experiments. It is often difficult to decide, however, exactly what is the best selection pressure to apply. If it is too low, a response may not be observed: but if it is too high, the reduction in genetic variation may lead to a reduction in response. Furthermore, the usually small effective population sizes used may lead to inbreeding depression (Rose, Graves & Hutchinson 1990). Thus, when resistance to pathogens has been selected for, cases of increased resistance are counterbalanced by studies reporting no evolution of resistance (Burges 1971; Briese & Podgwaite 1985). In particular, Lindfield's (1990) attempt to select artificially for increased resistance to the granulosis virus in *P. interpunctella* proved unsuccessful, with no increase in resistance after 10 generations.

The present study, by contrast, involves a laboratory natural selection experiment in which *P. interpunctella* has been allowed to evolve with its granulosis virus by establishing populations of the moth and the virus and maintaining them over a period of 2 years (Sait 1992). The selection pressure is therefore determined by the level of infection within the selected populations. Furthermore, in comparison with artificial selection experiments, natural selection experiments are less labour intensive and therefore allow a larger effective population size to be maintained. In addition, selection can take place over a longer period of time than is usually practical in artificial selection experiments (Rose, Graves & Hutchinson 1990). This approach does not allow a figure to be put on the extent of any correlations found — an aim of animal breeders — but any changes in fitness associated with the correlations can be quantified. Selection for resistance to microorganisms does not suffer from the problem associated with some laboratory natural selection experiments in which the actual pattern of selection may be unclear (Rose, Graves & Hutchinson 1990).

The laboratory conditions of the *P. interpunctella*/granulosis virus system closely simulate those found in nature. The system therefore constitutes an ideal model for the study of host–pathogen systems, with the experimental conditions likely to mimic some of the components of selection found in nature.

Materials and methods

In 1989 six populations of *P. interpunctella* were established in 23 cm × 23 cm × 11 cm perspex boxes, initially with 15 male and 15 female late fifth-instar larvae and 84 g of standard rearing medium, constituted from 400 g 'Farex' (Farley Health Products, Nottingham), 100 g dried brewer's yeast, 200 ml glycerol, 200 ml honey and 1 g of both sorbic acid and methyl paraban. In three populations an additional 12 fifth-instar larvae infected with granulosis virus were added. All six populations (three infected, three control) were maintained in the same incubator at 28 ± 1°C and 70 ± 5% r.h. (ranges) for 24 months, during which time the selected populations sustained their infection and the control populations remained virus free (Sait 1992). Host population size was monitored twice per week, while the level of infection was monitored and one-sixth of the food changed weekly.

After this period, adults which emerged from the discarded food of the control and selected populations were mated and allowed to lay eggs onto a standard rearing medium. The populations could not be sampled destructively at this stage because their population dynamics continued to be monitored. Due to the small number of adults produced from the discarded food of each of the populations, it was necessary to combine individuals from each of the three replicates. In this way selected and control lines were established. The two lines were then cultured separately for two generations on the standard rearing medium. To obtain larvae for bioassays, adults were first removed from the cultures and discarded. On each of the next 3 days, newly emerged adults were removed and allowed to mate and then lay eggs onto a plastic tray containing a small amount of food. By allowing two generations to pass before bioassays, the risks of confounding maternal effects, as well as the possibility of direct infection of larvae from the selected populations, were avoided. By using only newly emerged adults, confounding effects due to older females laying faster developing larvae (Lindfield 1990) were avoided.

First-day third-instar larvae were removed from the food for virus bioassays (for details see Sait 1992). Larvae at a standard developmental stage, rather than of a particular age, were chosen to avoid any differences in development rate confounding effects on relative resistance to the virus. Third-instar larvae provide the best compromise between ease of handling and ease of infection. Larvae were examined for presence of viral infection in the fourth and fifth instars. Virally infected larvae are easily distinguished by their opaque white coloration. Infected larvae do not pupate.

At the same time as the larvae required in the bioassays were removed, similar larvae were transferred to Petri dishes containing rearing medium and allowed to develop. The times to pupation, emergence and death of the adults were recorded along with the pupal weights. As adults, they were kept in individual pairs in small plastic pots and allowed to mate. The number of eggs laid and their subsequent viability were recorded.

In the light of the results of the first experiment, a further experiment was carried out after two more generations. Adults from the selected and control lines laid eggs into an empty plastic tray as before. Individual eggs were then transferred to individual cells within Petri dishes containing a small amount of food. The larvae were then observed daily. The day on which the larvae reached each instar was recorded.

Results

Dose mortality data were analysed using probit analysis rather than the exponential model proposed by Peto (1953). Huber & Hughes (1984) discuss the relative merits and assumptions of each model and suggest general guidelines for their use. In our bioassay we do not assume that all individuals are equally susceptible (cf. the exponential model) and since the slopes of the dose mortality regressions are less than 2 (Table 1), probit analysis is the most appropriate method. In probit analyses of the virus bioassays (Table 1; Fig. 1a, b), the lines for both stocks had non-significant χ^2 values, indicating satisfactory fits of the data to the probit lines (Finney 1971). The selected stock was more resistant than the control stock: the selected line (Fig. 1) lay below the control line for all mortality levels, and the lines were

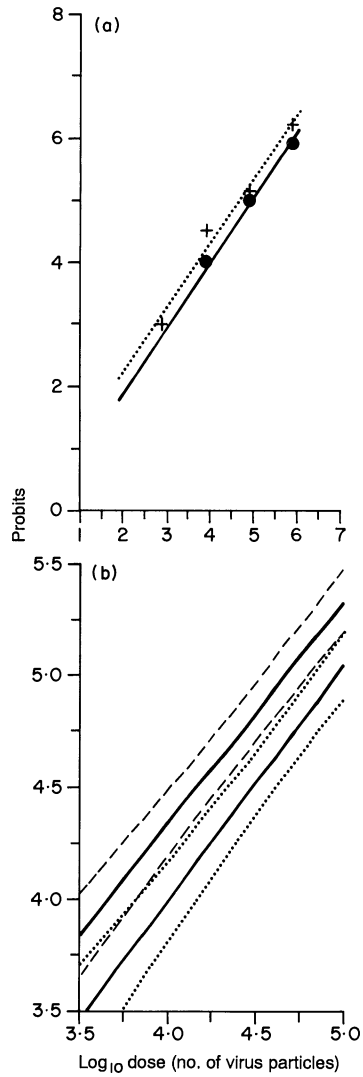

Fig. 1. (a) Probits in relation to log dose for the selected (●) (——) and control (+) (·····) lines generated from a probit analysis of bioassays with the virus. (b) Probits in relation to log dose showing the region of significant difference between the selected and control lines. Fiducial limits are shown for the selected (·····) and control lines (– – –).

significantly different, as judged by non-overlap of 95% fiducial limits, at LD_{25} and LD_{50} (Table 1) and came close to significance elsewhere. The resistance factor, the ratio of LD_{50} values (Burges 1971) was 1·96. The slopes of the lines were similar. A genuine, albeit small, increase in resistance has evolved, rather than the more susceptible individuals of the

Table 1. Probit analysis of bioassays with granulosis virus of selected and control populations of *P. interpunctella* (* significant differences between log doses based on non-overlap of 95% fiducial limits). Slope and intercept based on an arbitrary scale, log doses based on number of virus particles ingested

Population	Slope	Intercept	χ^2	Probability	Log LD_{05}	Log LD_{25}	Log LD_{50}	Log LD_{75}	Log LD_{95}
Selected	1·055	2·813	4·675	0·0966	3·405	4·325*	4·964*	5·603	6·523
Control	0·994	3·232	4·356	0·1133	3·016	3·992	4·671	5·350	6·326

population having simply been removed from the population (Burges 1971).

For comparisons of the life-history variables [carried out using the general linear models (GLM) procedure of SAS using type III sums of squares due to the occurrence of missing values in the data set], plots of residual against fitted values for each of the variables suggested normal distributions. All the analysis was consequently carried out on untransformed data.

Insects from the selected populations showed a much increased larval development time (egg to pupation) in both males and females (Table 2). On average they were 24% slower in developing and, in addition, females were significantly slower in developing than males. On the other hand, as judged by pupal weight, individuals from the selected populations were significantly larger (on average 5%) than those from the control populations (Table 2). Nonetheless, there was no significant difference between the two populations in the total number of eggs laid (Table 2). Individuals from the selected population, however, did have a significantly higher egg failure rate (Table 2).

The large increase in the larval development time of the selected populations, and the increase in egg failure rate, can both be regarded as trade-offs with the increased resistance to the pathogen. By contrast, individuals from the selected, more resistant population were on average larger than those from the less resistant control population. Hence there was an apparent trade-off between size and development rate (and between size and egg viability).

Table 3 shows that after two further generations the development rate differences between the two lines had diminished considerably. Nonetheless, the table indicates that the differences occurred only in the fourth and fifth instars: approximately 1 day and 2–3 day differences respectively. The fourth instar is highly resistant to the virus, and the fifth instar apparently totally resistant (Sait 1992). Thus the selected line developed equally rapidly through the more susceptible life stages, and the control line therefore gained no benefit in resistance terms from its more rapid development.

Calculation of fitness in the absence of the virus

As eggs are laid over a short period of time, we can assume that they are all laid at the mid-point of the adult lifespan (Howe 1953). With this assumption, the egg to egg period (t) was 47·9 days (SD 6·5) in the selected line and 41·1 days (SD 2·8) in the control line. If n eggs are laid of which S survive to become adults then fitness is given by Sibly & Calow (1986) as:

$$F=1/t.\log e.(n.S/2) \qquad \text{eqn 1}$$

The fitness of the selected line was 0·094 (where n = 190·0 − 5·1 = 184·9) while that of the unselected line was 0·111 (where n = 193·7); S is taken to be 1 as there was negligible mortality under the experimental conditions. There was therefore a 15% reduction in fitness in the selected line in the absence of the virus. It is interesting to note that after two further generations in the absence of the virus (Table 3), comparable calculations with the egg to egg period suitably altered suggest that the fitness reduction had diminished to 8%.

Fitness in the presence of the virus

At what level of infection with the virus, then, would there be a fitness benefit for the resistant population? In order to address this question we need to assume that the resistant and susceptible populations have the same development time, fecundity and egg viability in the presence of the virus as in the virus-free environment; that is, we need to ignore any possible effects of sub-lethal infection. At equal fitness, equation 1:

$$S_r/S_s=e^{1/ts.}\, n_s/e^{1/tr.}\, n_r \qquad \text{eqn 2}$$

Table 2. Results of the comparison of the life-history traits of the selected and control populations (significance level of the F-value, * < 0·05, ** < 0·01, *** < 0·001 where n is the sample size)

Character	Sex	Population (n)	$\bar{x} \pm SD$	Source	F-value
Development time (days)	Male	Selected (51)	34·9 ± 7·5 days	Population	87·89***
		Control (68)	28·4 ± 2·9 days		
	Female	Selected (66)	37·3 ± 7·4 days	Sex	6·54*
		Control (55)	29·8 ± 3·9 days		
Pupal weight (mg)	Male	Selected (51)	12·88 ± 2·05 mg	Population	6·22*
		Control (68)	12·05 ± 2·25 mg		
	Female	Selected (66)	17·56 ± 2·34 mg	Sex	271·9***
		Control (55)	16·94 ± 2·26 mg		
Eggs laid		Selected (26)	190·0 ± 67·8	Population	0·28
		Control (31)	196·0 ± 57·5		
Eggs failed		Selected (26)	5·1 ± 5·4	Population	10·61**
		Control (31)	2·3 ± 3·9		

Table 3. Development times (days) to each larval stage for control and selected populations (significance level of difference between selected and control, *t*-tests, ** $P < 0.001$) where (n) is the sample size

Instar	Population (n)	Mean no. of days to reach instar (± SD)		Mean no. of days within instar (± SD)	
		Males	Females	Males	Females
Second	Selected (73)	8·6 ± 0·5	8·59 ± 0·55	3·0 ± 0·4	3·0 ± 0·4
	Control (65)	8·5 ± 0·8	8·74 ± 0·62	3·0 ± 0·5	3·0 ± 0·5
Third	Selected (69)	11·6 ± 0·5	11·65 ± 0·64	3·0 ± 0·5	3·1 ± 0·5
	Control (62)	11·5 ± 0·9	11·73 ± 0·71	3·2 ± 0·5	2·9 ± 0·4
Fourth	Selected (67)	14·6 ± 0·6	14·78 ± 0·79	5·0 ± 1·3**	5·2 ± 0·8**
	Control (60)	14·7 ± 0·9	14·65 ± 0·73	4·1 ± 0·6	4·1 ± 0·7
Fifth	Selected (67)	19·6 ± 1·2**	20·0 ± 0·9**	9·9 ± 2·8**	10·5 ± 2·8**
	Control (60)	18·8 ± 1·1	18·78 ± 0·80	7·7 ± 2·5	8·5 ± 1·7
Pupation	Selected (67)	29·5 ± 2·6**	30·5 ± 2·7**		
	Control (60)	26·5 ± 2·4	27·2 ± 1·7		

Thus

$$S_r = 1{\cdot}052 * S_s \qquad \text{eqn 3}$$

where subscripts refer to resistant and susceptible populations. Therefore, for the resistant morph to gain a fitness advantage in the presence of the virus, its survival would have to be more than 1·052 times greater than that of the susceptible morph. It is possible to gain some impression of how likely this is from the bioassay results already presented (though, of course, naturally the virus would not be presented to the host as a single inoculum at a single point in their life). Figure 1a indicates that the criterion is fulfilled up to a log dose of greater than 5·99 virus particles. That is to say, the resistant morph would be favoured unless the mortalities due to the virus were very high (86% in the selected line, 90·5% in the control line).

Discussion

The results have shown that exposure to the virus has led to the evolution of increased resistance in the host. This resistance has been bought at a cost in fitness terms due to an increase in larval development time and reduced egg viability. The laboratory evolutionary approach has thus been successful in selecting for increased resistance. Evolutionary experiments are a useful tool in the study of correlations between life-history traits because their efficient use of laboratory resources allows larger effective population sizes to be maintained over longer periods of time than in artificial selection experiments. In addition the selection pressures are determined by the host–pathogen interaction and are therefore likely to reflect selection pressures encountered in nature.

It has been shown (Sait 1992) that sub-lethal infections of the granulosis virus in *P. interpunctella* consistently affect only male and female fecundity and egg viability. There is no evidence of sub-lethal effects consistently increasing development time (nor pupal weight). Furthermore, neither the control insects from the virus bioassays nor those used in the examination of the life-history traits showed any sign of infection with the virus. It therefore seems certain that the differences between the selected and control lines were due to evolved characteristics and cannot be explained by a sub-lethal infection.

The positive correlation between pupal size and resistance to the pathogen seems likely to have been in part due to the commonly observed covariance between development time and size (Stearns & Koella 1986). In this view, the positive correlation with size is a part of a wider overall genetic trade-off with resistance, which has produced negative correlations with other characters. It is difficult to quantify the effect of the increased size of the resistant morph in fitness terms. Size is often positively correlated with fecundity (Smith 1991). However, in our assessment of fecundity there was no significant difference between the stocks. It is reasonable to assume that size might affect survival. However, our experimental design allowed negligible mortality in the assay experiment. Nonetheless, in their natural environment the resistant morphs may have a smaller fitness cost than that calculated, due to benefits from their increased size not realized under the present experimental conditions.

When invertebrate populations, from both the wild and the laboratory, have been compared, large differences in their resistance to viruses have been found (Burges 1971; Briese & Podgwaite 1985). In Vail & Tebbets' (1990) comparison of six wild and two laboratory populations of *P. interpunctella*, resistance factors were as great as 3·8. Furthermore,

Hunter & Hoffmann (1973) found greater than a seven-fold difference between two laboratory strains of Indian meal moth. The resistance factor of 1·96 in the present study is modest in comparison. However, the calculations of fitness suggest that enough resistance has evolved to yield a net increase in fitness in the resistant population at the levels of mortality encountered in the infected populations. The resistance that has developed is an appropriate response to the selection pressure encountered, given the costs associated with that resistance. The prediction that these costs will lead to the loss of resistance when selection is relaxed is supported by the fact that the fitness cost in the selected line was reduced to 8% after two further generations in the absence of the virus. The development of resistance to microorganisms used in biological control may be crucial to the effectiveness and economic viability of such programmes. We have shown that in this system the development of resistance is constrained by trade-offs with other fitness characters. For this reason an appropriate level of resistance has evolved in response to the viral-induced mortality encountered. At higher selection pressures more resistance is likely to evolve, but it is unlikely to be any greater than that required to gain a fitness benefit in the presence of the virus. In addition, where trade-offs exist, evolved resistance is likely to be lost in the absence of the pathogen and hence periodic relaxation of the control measures may effectively manage the development of resistance.

The present results appear to contradict Hochberg, Michalakis & de Meeus' (1992) model, which demonstrates that a host may gain in fitness by reducing its time to reproduction in the presence of a parasite (shortening its target period). Here, by contrast, resistant individuals took more, not less time to develop. However, susceptibility to the virus is highly age dependent (Sait 1992). Virus mortality is concentrated in the early instars, with the fourth and fifth being practically not susceptible to infection. If the selected line had developed through the early vulnerable instars more slowly than the unselected line, then selected larvae may have been more susceptible than unselected larvae on a particular day of development, even though they were more resistant at a particular life stage, first day third instar. However, the increased development time became apparent only in the fourth and fifth instars, when the larvae are virtually immune. The selected line was thus unequivocally more resistant in the susceptible instars and thereby gained a fitness benefit in the presence of the virus. Our results therefore support and actually extend those of Hochberg, Michalakis & de Meeus (1992) by additionally taking account of the possible costs of resistance. They emphasize that if those costs include an increase in development time, then that increase might be expected to be concentrated in the least susceptible period of the host's life.

Acknowledgements

Michael Boots was supported by a Natural Environment Research Council studentship.

References

Bell, G. & Koufopanou, V. (1986) The cost of reproduction. *Oxford Surveys in Evolutionary Biology* **3,** 83–131.

Briese, D.T. & Mende, H.A. (1983) Selection for increased resistance to a granulosis virus in the potato moth, *Phthorimsaea operculla* (Zeller). *Bulletin of Entomological Research* **73,** 1–9.

Briese, D.T. & Podgwaite, J.D. (1985) Development of viral resistance in insect populations. *Viral Insecticides for Biological Control* (eds. K. Maramorosch & K.E. Sherman), pp. 361–398. Academic Press, New York.

Burges, H.D. (1971) Possibilities of pest resistance to microbial control agents. *Microbial Control of Insects and Mites* (eds. H.D. Burgess & N.W. Hussey), pp. 445–457. Academic Press, London.

Charlesworth, B. (1980) *Evolution in Age-Structured Populations*. Cambridge University Press, Cambridge.

Falconer, D.S. (1989) *Introduction to Quantitative Genetics*. 3rd edn. Longman, Harlow, Essex.

Finney, D.J. (1971) *Probit Analysis*. Cambridge University Press, Cambridge.

Granados, R.R. (1980) Infectivity and mode of action of baculoviruses. *Biotechnology and Bioengineering* **22,** 1377–1405.

Harvey, T.L. & Howell, D.E. (1965) Resistance of the housefly to *Bacillus thuringiensis*. *Journal of Invertebrate Pathology* **7,** 92–100.

Hochberg, M.E., Michalakis, Y. & de Meeus, T. (1992) Parasitism as a constraint on life-history evolution. *Journal of Evolutionary Biology* **5,** 491–504.

Howe, R.W. (1953) The rapid determination of the intrinsic rate of increase of an insect population. *Annals of Applied Biology* **40,** 134–150.

Huber, J. & Hughes, P.R. (1984) Quantitative bioassay in insect pathology. *Bulletin of the Entomological Society of America* **30,** 31–34.

Hunter, D.K. & Hoffmann, D.F. (1973) Susceptibility of two strains of Indian meal moth to a granulosis virus. *Journal of Invertebrate Pathology* **21,** 114–115.

Lenski, R.E. (1988) Experimental studies of pleiotropy and epistasis in *Escherichia coli* I: Variation in competitive fitness among mutants resistant to virus. *Evolution* **42,** 425–432.

Lindfield, S.M. (1990) *Microevolution in an insect–virus interaction*. PhD thesis, University of Liverpool.

Maynard Smith, J., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., Lande, R., Raup, D. & Wolpert, L. (1985) Developmental constraints and evolution. *Quarterly Review of Biology* **60,** 265–287.

Peto, S. (1953) A dose response equation for the invasion of microorganisms. *Biometrics* **9,** 320–335.

Rose, M.R. & Charlesworth, B. (1981a) Genetics of life history in *Drosophila melanogaster* I. Sib analysis of adult females. *Genetics* **97,** 173–186.

Rose, M.R. & Charlesworth, B. (1981b) Genetics of life history in *Drosophila melanogaster* II. Exploratory selection experiments. *Genetics* **97,** 187–196.

Rose, M.R., Graves, J.L. & Hutchinson, E.W. (1990) The

use of selection to probe patterns of pleiotropy in fitness characters. *Insect Life Cycles* (ed. F. Gilbert). Springer-Verlag, Berlin.

Sait, S.M. (1992) *The population dynamics of an insect virus interaction*. PhD thesis, University of Liverpool.

Sibly, R.M. & Calow, P. (1986) *Physiology Ecology of Animals: an Evolutionary Approach*. Blackwell Scientific Publications, Oxford.

Smith, R.H. (1991) Genetic and phenotypic aspects of life-history evolution in animals. *Advances in Ecological Research* (eds. M. Begon, A.H. Fitter & A. Macfadyen). Academic Press, London.

Stearns, S.C. (1980) A new view of life-history evolution. *Oikos* **35,** 266–281.

Stearns, S.C. (1989) Trade-offs in life-history evolution. *Functional Ecology* **3,** 259–268.

Stearns, S.C. & Koella, J.C. (1986) The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. *Evolution* **40,** 893–913.

Vail, P.V. & Tebbets, J.S. (1990) Comparative biology and susceptibility of *Plodia interpunctella* (Leidoptera: Pyralidae) populations to a granulosis virus. *Environmental Entomology* **19,** 791–794.

Watanabe, H. (1967) Development of resistance in the silkworm *Bombyx mori* to peroral infection of a cytoplasmic polyhedrosis virus. *Journal of Invertebrate Pathology* **9,** 474–479.

Williams, G.C. (1957) Pleiotropy, natural selection and the evolution of senescence. *Evolution* **11,** 398–411.

Received 18 August 1992; revised 10 December 1992; accepted 15 February 1993