Fitness traits associated with the red back phenotype in the migratory grasshopper, *Melanoplus sanguinipes*

F. R. H. Hodson[1] and W. Chapco[2]

Department of Biology, University of Regina, Regina (Saskatchewan, Canada S4SOA2), 11 July 1985

Summary. The dominantly inherited variant, red back, in the migratory grasshopper, *Melanoplus sanguinipes*, is rare in nature. The present study illustrates that is this probably due, in part, to the trait's association (either through pleiotropy of the red back gene, *Pro* and/or closely linked fitness loci) with lower egg hatchability, greater developmental time, greater mortality during later stages, and a greater tendency to have an extra instar.

Key words. Melanoplus sanguinipes; grasshoppers; genetics; fitness; hatchability; polymorphism.

Red back is a dominantly inherited trait in the migratory grasshopper, *Melanoplus sanguinipes*; the underlying gene, symbolized as *Pro*, is relatively uncommon (about 0.4% on average) throughout North America[3,4]. The frequency is too low for the trait to be considered polymorphic, contrasting with that of tibia color, for example[4]. A variety of fitness components (egg number and pod production rate, egg hatchability, time-to-hatch, pre-reproductive mortality, number of molts prior to fledging, oviposition site preference and diurnal coupling behavior) were measured in red back and control strains. If substantial, differences in at least one of these components could account for the trait's low occurrence. The findings are presented here.

Materials and methods. The control stock in these experiments is a nondiapausing laboratory strain. The red back strain originated in the wild, but was subjected to two generations of backcrosses to the control followed by selection for the marker; it is now a well established continuously breeding stock. Presumably, the genetic background of the red back strain is about 75% similar to that of the control strain. Rearing conditions have been previously given[3]. Methods for determining fitness parameters are described in the following three experiments. 1) Egg pods that had been deposited in moist sand were removed daily and placed in glass vials (85 × 23 mm) containing moistened vermiculite; vials were then sealed with plastic wrap and incubated at 30 °C (a series of vermiculite-water concentrations was originally set up to examine possible differential effects on a number of egg parameters; since none were found, this point will not be mentioned again). Hatchlings were counted daily as they emerged. The vials were kept for at least 1 week after the last eclosion (the normal hatching time is about 21 days), pods removed, and unhatched eggs scored. Hatchability was calculated as a percentage of eggs hatched and pod incubation time was defined as the time of hatching of the first emerging larva. 2) In this experiment, strain differences with respect to mortality and number of instars prior to fledging were examined. Egg pods were collected from control and red back breeding cages within 24 h of oviposition and incubated in moisted vermiculite at 30 °C until hatching occurred. Five first instars were collected from each pod within 24 h of the first eclosion and singly placed in a capped plastic vial (4.5 × 9.8 cm). On reaching the fifth instar, survivors were individually placed into 500 ml glass sealers (8 × 10 cm) closed with screened lids. Containers were checked daily for exuviae and the individuals scored up to and including 1 week after reaching the adult stage. 3) Possible reproductive behavioral and physiological differences were examined in this study. Replicate cages containing 6–8 pairs of breeding individuals of each strain were established and maintained for approximately 30 days. Two tubes of moistened sand were placed in each cage for ovipositioning: one containing 'wet sand' (10:1 sand to water) and the other containing 'dry sand' (20:1 sand to water). The cages provided holes for insertion of two sand tubes, front and back – the latter being proximal to a 25 W incandescent bulb. The positions of the wet and dry sand tubes were alternated daily resulting in four treatment combinations. Recorded for each cross were the numbers of males and females present at a given time, the number of couplings at three times of each day (09.00, 12.00 and 16.00 h), and the number of egg pods laid into each substrate type. The procedure allowed for estimation of the rate of egg pod deposition as well as a re-examination of some of the parameters obtained in part 1.

Results and discussion. All means and percentages, measures of variation (based on analysis of variance: MSE's or mean square errors and MS-reps w. stains or mean squares between repetitions within strains, in the case of nested designs), and tests of significance (F tests for Anovas and G or likelihood ratio tests for frequency data[5]) are laid out in the table. Analysis of variance was performed on arc-sine transformed hatchability proportions. Mean squares and degrees of freedom are based on larger sets of data involving strains not of interest here. This was done to increase the power of the tests performed. It can be seen that there is no significant difference between the number of eggs per pod of red back and control strains. With respect to rate of egg pod deposition (expressed on a per-female basis, corrected for female mortality and then log-transformed to stabilize the

Means and tests of significance for several fitness traits in a comparison of red back and control strains of *Melanoplus sanguinipes*

Trait	Means		MSE (df)	MS-reps	F	G (df)
	Red back	Control		Wed strains (df)		
Egg no. (exp. 1)	13.9 (27)	14.9 (50)	18.61 (290)	–	1.49	
Egg no. (exp. 3)	12.0 (62)	13.5 (101)	13.45 (254)	130.0** (6)	0.57	
Egg pod deposition rate	0.1023	0.1362	2.62 (18)	–	4.5*	
% Hatch (exp. 1)	46.5 (26)	83.1 (40)	259.1 (220)	–	48.0**	
% Hatch (exp. 3)	37.1 (62)	78.1 (101)	304.9 (254)	1916.5** (6)	15.7**	
Pod incubation time	21.6 (21)	20.2 (37)	0.57 (209)	–	42.2*	
% Mortality: 1st instar	15.0 (40)	13.5 (37)				0.03 (1)
2nd–4th instar	10.3 (32)	3.3 (31)				1.05 (1)
5th instar-1-wk adult	34.6 (26)	10.3 (29)				4.57* (1)
% Insects with 6 instars	65.6 (32)	6.45 (31)				27.7** (1)
% Coupling at 9 h	69 (26)	57 (131)				
12 h	23	28				
16 h	8	15				1.59 (2)
Substrate choice:						
% Pods in wet sand	65.7 (35)	70.4 (71)				0.24 (1)
% Pods in cage front	37.1 (35)	40.9 (71)				0.13 (1)

*$p < 0.05$, **$p < 0.01$.

variances[1]), there is a significantly lower rate for the red back strain. One of the most noticeable effects of the *Pro* locus has to do with hatchability. The trait's value is about 40% units below that of the control. a highly significant difference. In the third experiment, the effect is so large as a mean square, that it exceeds the 'between repetitions within strains' mean square term. With respect to development time, pods from the dominant strain take about 1.4 days longer to hatch than those from the control strain, a significant difference. There is no evidence that mortality is higher once the grasshoppers have emerged, that is, up to the fourth instar. From the fifth instar to 1 week post-fledging, there is a significantly higher mortality of red back insects. *M. sanguinipes* normally go through five instar stages[7]. This study shows that about ⅔ of red back individuals have an extra instar ('inserted' between the normal third and fourth instar stages); this proportion is highly significantly greater than that of the control strain. Both strains display statistically similar diurnal coupling distributions and oviposition site preferences. If one can extrapolate from these laboratory findings, it is likely that red back's rarity in nature is due, in part, to its association with low hatchability, longer hatching time and greater mortality during the later stages. Variation in the number of nymphal stages occurring prior to fledging is common in species of grasshoppers and locusts[7], including *M. sanguinipes*[8]. While temperature, nutrition, and crowding are important influencing factors[9–11], the present result (the first for an acridid) reveals a genetic connection as well. According to Pfadt et al.[8], adult weight and development time of (non-red back) 6-instar grasshoppers tend to be greater than those of 5-instar individuals (data not presented here suggest the same is true for red back variants). These features could allow a species to increase its habitat range, as appears to be the case in *M. femurrubrum*[6]. But red back is rare everywhere[4], notwithstanding a couple reports of local populations with high incidences of the morph[12]. Any benefits to red back that a supernumerary stage might accrue (perhaps in more southern areas), are obviously balanced by other factors. In northern climates, however, having an extra instar would have to be considered disadvantageous.

It could be argued that the effects detected are not due to the *Pro* locus itself but are the result of loci elsewhere in the red back stock's genome. It will be recalled, however, that the variant strain in its development received an infusion of genes from the control strain, thereby lessening the possibility of background effects, certainly those associated with loci on other chromosomes (2n ♂ = 23 in this species). Nonetheless, there may still be present in the red back strain, genes from nature that are closely linked to the *Pro* locus, and that are, in part, responsible for the effects revealed here and perhaps the trait's low incidence. The latter would require, of course, that the *Pro* locus and such genes be in linkage disequilibrium[13]. While this possibility cannot be discounted, it should be noted that thus far, population studies have revealed no evidence of this phenomenon in *M. sanguinipes* at least with respect to color and electrophoretic traits[4,14]. In any case, it cannot be unequivocally stated that the fitness disturbances are solely the result of pleiotropy on the part of the *Pro* gene.

1 A part of an M.Sc. thesis of F.R.H. Hodson, Univesity of Regina (1985).
2 Financial assistance from the Natural Sciences and Engineering Research Council (A0485) is greatfully acknowledged. Address reprint requests to W.C.
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0014-4754/86/040444-02$1.50 + 0.20/0

Intracolonial worker relationship and sperm competition in the honeybee (*Apis mellifera* L.)

R. F. A. Moritz

Institut für Bienenkunde, J.-W. Goethe-Universität Frankfurt, Fachbereich Biologie, Karl-von-Frisch-Weg 2, D-637 Oberursel (Federal Republic of Germany), 8 March 1985

Summary. Queens, homozygous for three marker genes, were inseminated with eight different types of semen, each carrying one combination of the markers. Intracolonial worker relationship ($\hat{r}_c$) was estimated by genotype frequencies in the offspring of the experimental queens. $\hat{r}_c$ was larger than under random distribution of semen types in the spermatheca. Estimates of most quantitative genetical parameters will be more accurate using the presented estimator for intracolonial relationship. The insemination order affected genotype frequencies in the offspring, suggesting a weak 'last male advantage'. There was, however, no evidence of intra spermathecal sperm competition.

Key words. Apis mellifera; sperm competition; relatedness.

The problem of relatedness of workers in colonies of social hymenoptera and its consequences for the evolution of social behavior and the mechanisms of kin recognition, has been addressed many times since the stimulating papers of Hamilton[1,2]. In honeybees, however, polyandry weakens the arguments of kin selection as the average intracolony relationship between workers is low. Recently it has been repeatedly shown[3,4] that semen of different drones is well mixed in the spermatheca of the queen and that there is no clumping of semen as claimed by others[5,6]. However, the drones which were used for insemination did not contribute equally to the offspring, which increased the average intracolonial relationship. This has not only consequences for socio biological theories[4], but also affects important estimators in quantitative genetics. In studies which estimate heritabilities of worker characters mostly equal contributions of each drone were assumed[7,8].