

Evolutionary stability since the  
Pleistocene illustrated by reproductive  
compatibility between Swedish and Spanish  
*Helophorus lapponicus* Thomson (Coleoptera,  
Hydrophilidae)

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Spanish and Swedish populations of *Helophorus lapponicus* are shown on fossil evidence to have been isolated from one another for about 10 000 years, since the end of the Pleistocene. When cross-bred they show some evidence of heterosis in the F<sub>1</sub>, with reduced viability in the F<sub>2</sub> and back-cross generations. The level of compatibility of these populations is similar to that found when different populations of Lepidoptera species are crossed, and quite different from the incompatibility revealed by studies of interspecific hybridization. The degree of evolutionary stability revealed is consistent with the apparent absence of evolution revealed by studies of Pleistocene fossil Coleoptera.

KEY WORDS:—Evolution – Pleistocene – *Helophorus* – Coleoptera – Hydrophilidae.

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INTRODUCTION

Among the most startling of the phenomena revealed by the work of G. R. Coope and his colleagues at Birmingham University on Pleistocene fossil Coleoptera are the absence of morphological differences between Pleistocene fossil material and modern specimens of the same species, and the enormous changes that have taken place in the ranges of many species (Coope, 1970). While the changes in distribution of the various species are demonstrated by accurate identification of modern and fossil specimens, the absence of evolutionary change, and in particular of speciation, implied by the morphology of the fossils, is more controversial. Since insect taxonomy is based almost entirely on details of the chitinous exoskeleton, and it is these fragments that are preserved as fossils, the morphological study of the Pleistocene material is able to use the same structures

that form the basis of taxonomic work on modern material (Coope & Angus, 1975). However, it may be argued that no demonstration of the absence of morphological change, however sophisticated, can rule out the possibility of physiological differences between Pleistocene forms and their modern counterparts. Only the fact that the species assemblages found in Pleistocene deposits are consistent with what is known about the present day ecologies of the species concerned suggests that they have not changed their ecological requirements and that their physiologies must have been similarly constant.

An alternative approach to the problem is provided by breeding experiments involving populations which on taxonomic grounds belong to the same species, but are geographically isolated from one another. Such isolated populations become even more appropriate for investigation if the timing of the initial isolation can be estimated. One species which is suitable for such studies is the hydrophilid water beetle *Helophorus lapponicus* Thomson. The main part of its range extends from the northern half of Scandinavia across European Russia to eastern Siberia and Mongolia, but it has isolated populations on the island of Öland in the Baltic, in the Cantabrian mountains of northern Spain, and in the Transcaucasus (Angus, 1974). I have also taken it in the Sierra Guadarrama in central Spain.

The Spanish populations are of particular interest because they lie in the general area of western Europe for which some climatic (and therefore faunal) inferences can be drawn from studies of British Pleistocene Coleoptera. *Helophorus lapponicus*, as indicated above, is not a British species today, but it has lived here at various times during the last (Würm, Weichselian or Wisconsinan) glaciation. The earliest known occurrence is in the Higher Tattershall Silts at Tattershall Castle Pit, Lincolnshire, from where I have seen a number of well-preserved pronota (Fig. 1A). These silts contain an extensive and largely thermophilous fauna, in which the presence of *H. lapponicus* and some other water beetles is attributed to the formation of cold grassy pools from melting snow in the spring (Girling, 1974, 1980). They are correlated with the deposit at Isleworth, Middlesex, described by Coope & Angus (1975), and have been radiocarbon dated at  $43\,000 \pm^{1400}_{1100}$  years BP (Birm 431) (Girling, 1974). A more recent, and therefore more important, occurrence is provided by a single pronotum from silts recovered from the

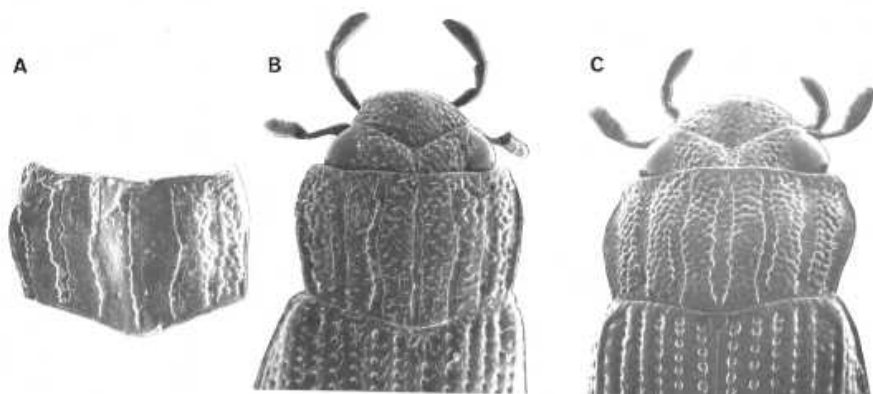


Figure 1. Scanning electron micrographs of modern and fossil *Helophorus lapponicus*,  $\times 27$ . A, Fossil pronotum from Tattershall, Lincolnshire; B, head and pronotum of a modern specimen from Karasuk, western Siberia; C, head and pronotum of a modern specimen from northern Spain. The head and pronotum of C are slightly deflexed.

excavations for the Farmoor Reservoir, near Oxford. The silts have been radiocarbon dated at  $10\,600 \pm 240$  years BP (Birm 590), which refers them to the final cold phase of the Last Glaciation. The fauna they contain is typical of that period (Coope, 1976), during which arctic beetle faunas have been found in Britain as far south as Cornwall (Coope, 1977). Thus, at this time (between 11 000 and 10 000 years BP) *H. lapponicus* is likely to have occurred in lowland areas of western France. The post-glacial (Flandrian) period began abruptly at about 10 000 years BP, and two extensive beetle faunas, one from the English Midlands (Osborne, 1974) and one from south-west Scotland (Bishop & Coope, 1977), dated at about 9500 years BP contain neither *H. lapponicus* nor any other species with northern or eastern distributions today. These faunas and all subsequent ones indicate that the climate in western Europe has been broadly similar to that existing today for the last 10 000 years, and this, in conjunction with the rapidity with which the distributions of Coleoptera have been shown to respond to climatic change (Coope, 1977) suggests that the western populations of *H. lapponicus* have almost certainly been confined to their present ranges for the past 10 000 years.

#### MATERIAL AND METHODS

Spanish *H. lapponicus* were collected on 27 and 28 March 1979, near La Salceda (Provincia de Segovia) and near Areños and Aguilar de Campoo, both localities near the headwaters of the Rio Pisuerga in the Provincia de Palencia. Living beetles were brought home and set up in an outdoor insectary in plastic boxes, following the procedure described by Angus (1973), except that since *H. lapponicus* places its egg cocoon among submerged vegetation, no earth bank for oviposition was provided. The beetles commenced breeding without delay, and virgin females were reared for further experiments.

The Swedish *H. lapponicus* were collected by Dr L. Huggert of Umeå University, on 13 June 1979 at Djupgroven, Varuträsk, west of Skellefteå in the province of Västerbotten. They were one female and two males. The female proved to be beyond breeding age, but the males were placed with virgin Spanish females, which bred successfully.

Details of my method of rearing *Helophorus* are given by Angus (1973). *Helophorus lapponicus* lays its eggs in batches of about six, in silk cocoons. These were harvested daily and placed in plastic pill boxes, on damp filter paper. At room temperature (about 20°C) the eggs hatch in about a week. The larvae were fed on fresh *Tubifex* worms, and 24 h after hatching they were transferred to individual pill boxes, to avoid cannibalism. The larvae moult twice, and are ready to pupate after about 14 days. Pupation was in banks of damp sand in the pill boxes, and adult emergence normally takes place after a further 14 days. Under favourable conditions the adults start breeding 2–3 weeks after emergence, so that each generation takes about 2 months from being laid as an egg to starting to breed. In the field there is one generation per year.

In order to avoid the effects of winter and the shortening day length in autumn, the adult beetles were kept in a Fisons controlled environment cabinet, set at 20°C and 15 h daylight.

Table 1. Breeding experiments with Spanish and Swedish *H. lapponicus*

	Eggs	1st stage larvae	Fully grown larvae	Adults	Survival %	Sex ratio ♀:♂
Control:						
La Salceda ♀ × ♂	24	24	15	2	8.3	1:1
A. de C.* ♀ × ♂	20	20	10	2	10	0:2
Areños, ♀ × ♂						
Brood 1	8	8	6	2	25	2:0
Brood 2	10	10	7	2	20	2:0
F <sub>1</sub> :						
Areños ♀ × Sweden ♂	33	33	24	17	51.5	8:9
A. de C. ♀ × Sweden ♂	32	32	20	8	25	5:3
F <sub>2</sub> :						
(Areños × Sweden) ♀ × ♂	46	16	13	6	13	3:3
(A. de C. × Sweden) ♀ × ♂	4	0	0	0	0	—
(Areños × Sweden) ♀ × (A. de C. × Sweden) ♂	7	3	1	0	0	—
Back-cross:						
(Areños × Sweden) ♀ × Sweden ♂						
Brood 1	40	10	3	1	2.5	1:0
Brood 2	21	4	2	2	9.5	1:1
(Areños × Sweden) ♀ × A. de C. ♂						
Brood 1	105	105	70	20	19	13:7
Brood 2	15	8	4	1	6.7	1:0
(A. de C. × Sweden) ♀ × A. de C. ♂	28	17	7	4	14.3	3:1
Out-cross:						
La Salceda ♀ × (Areños × Sweden) ♂	10	10	3	1	10	1:0
F <sub>3</sub> :						
♀ (Areños × Sweden) ♀ × ♂ A. de C. × A. de C. ♂	20	10	5	2	10	1:1
♀ (A. de C. × Sweden) × ♂ A. de C. ♀ × ♂	17	12	2	1	5.9	1:0

\* A. de C.—Aguilar de Campoo.

## RESULTS

The various crosses performed, with the number of broods in each case, and the results obtained from them, are listed in Tables 1–4. It will be seen that Spanish *H. lapponicus* suffered high mortality when reared in the laboratory. This high mortality has been consistently encountered with Spanish *lapponicus*, so that the

control results may be taken as typical, despite the small sample size. With many British *Helophorus* species I have achieved over 75% survival from egg to adult (Angus, 1973), but there are some species, such as *H. glacialis* Villa, which I have been completely unable to breed. *Helophorus lapponicus* thus comes between the two extremes.

The  $F_1$  crosses show a distinct improvement in survival. Comparison of brood survival gives a result significant at the 90% level, but a  $2 \times 2$  contingency table gives a much greater significance. Although use of pooled data is justified by the similarity of brood size within each cross, the high significance is due to the Areños  $\times$  Sweden cross. The Aguilar de Campoo cross shows a scarcely significant increase in survival, whereas comparison of survival in the two  $F_1$  crosses shows the Areños cross to have an increased survival significant at the 95% level. The poor survival of the controls probably reflects unsuitability of laboratory conditions to the Spanish stock. It is possible that Swedish beetles would have survived better, so that the improved survival of the  $F_1$  could be the result either of heterosis or of improved tolerance of laboratory conditions inherited from the Swedish parent.

The level of mortality of the  $F_2$ ,  $F_3$  and back-cross broods is similar to that of the control, with some suggestion of reduced survival in the  $F_2$  and the back-cross to the Swedish male. In the latter case the male was old and may have been running out of sperm. Spermatogenesis in this species is complete after about 1 month, and old males do run out of sperm. It will be seen that failure of eggs to hatch was a major source of mortality in these broods and larval and pupal survival was in fact marginally greater than in back-crosses to Spanish males.

A feature of all the crosses after the  $F_1$  is embryonic abortion. All egg cocoons were opened either after the larvae had hatched, or after 10 days, and in many cases eggs showed some embryonic development, which had apparently stopped after 2–3 days. Unfertilized eggs tend to rot after a week or so, and this decay affects other eggs in a cocoon, so that exact numbers of embryonic abortions could not be ascertained. This type of failure was not found in either control or  $F_1$  cocoons, nor have I found it in other *Helophorus* species. It must therefore be regarded as evidence of reduced compatibility between the two populations.

The  $F_3$  generation was affected by difficulties with the controlled environment cabinet, but shows that the beetles were still able to breed successfully.

The back-cross to Spanish males shows a significant reduction in the proportion of male offspring, especially when the numbers reared from individual cocoons are analysed. Normally laboratory reared broods of *Helophorus* contain almost equal

Table 2. Mortality comparisons. *t*-Tests using percentage survival per brood (variances of the different crosses not significantly different by Bartlett's test)

Cross	<i>t</i>	d.f.	<i>P</i> (2-tailed)
Control v. $F_1$	-2.224	4	0.0902
Control v. $F_2$	1.927	5	0.112
Control v. Back-cross	1.129	7	0.296
Control v. Back-cross (Swedish ♂)	1.541	4	0.198
Control v. $F_3$	1.284	4	0.268

Table 3. Mortality comparisons. Chi-square  $2 \times 2$  contingency tables using pooled data for each cross

Cross	<i>P</i>
Control v. $F_1$	0.0005
Control v. $F_1$ (A. de C. $\times$ Sweden)	0.106
$F_1$ (Areños $\times$ Sweden) v. $F_1$ (A. de C. $\times$ Sweden)	0.028
Control v. $F_2$	0.116
Control v. back-cross	0.865
Control v. back-cross (Swedish ♂)	0.149
Control v. $F_3$	0.527

numbers of males and females, so that there does seem to be lowered viability of the males (heterogametic sex) in this cross.

The average size of the beetles was not affected by the crosses, being about 3.5 mm in all cases. Spanish *H. lapponicus* (Fig. 1C) tend to have the pronotum a little broader, with wider marginal grooves, and more granulate than Swedish specimens, which resemble the Siberian one shown in Fig. 1B. The hybrids were intermediate in these features, with the back-crosses to Spanish males tending to re-establish the Spanish facies. Chromosomally the two populations appear to be identical. Chromosome preparations from developing embryos, using the method of Crozier (1968), but with Giemsa staining, showed that both Spanish and hybrid specimens have  $2N = 20 + XY$  (♂),  $XX$  (♀), and that there is no morphological difference between the chromosomes of the two populations.

#### DISCUSSION

It is clear from these experiments that Spanish and Swedish *H. lapponicus* are genetically different from one another, and that this results in reduced compatibility between them. However, it is also clear that there is no reproductive barrier between them, so that it is most likely that if these populations were to be brought together in nature, as a result of climatic change, they would merge genetically.

The environments of the Spanish and Swedish populations are obviously different from one another, both in terms of physical features such as daylength change and the timing of the seasons (Swedish beetles would not be active in March), and also in terms of the animals and plants present. It is thus inevitable that there will be genetic differences between the beetles, and it would have been

Table 4. Sex ratio of the back-cross. No. ♂ v. No. ♀ using paired sample *t*-test

	<i>t</i>	d.f.	<i>P</i> (2-tailed)
Brood	-1.761	2	0.107
Cocoon	-2.153	17	0.023

very surprising had no heterosis or incompatibility been revealed when they were crossed.

The results of crossing these populations of *H. lapponicus* may be seen in perspective when compared on the one hand with the effects of hybridizing *H. lapponicus* with the related *H. minutus* F., and on the other hand with experiments involving crossing different populations within other insect species.

I have hybridized Spanish *H. lapponicus* with English *minutus* on a number of occasions in an attempt to investigate the apparent morphological intergradation between these species in western Siberia (Angus, 1974). The F<sub>1</sub> hybrids have a much lower mortality than Spanish *lapponicus* in the laboratory, though in this case the effects of heterosis are less striking as *H. minutus* also has low mortality. However, these F<sub>1</sub> hybrids also show a certain amount of embryonic abortion. Although *H. minutus* has the same chromosome number as *lapponicus* there are morphological differences between the chromosomes of the two species. Nevertheless, spermatogenesis in the hybrids appears normal. No F<sub>2</sub> or back-cross generations have been obtained, but hybrid males have mated successfully with female *lapponicus*, and male *minutus* has crossed with female hybrids. In both cases the result was embryonic abortion.

The effects of crossing isolated populations within species have been investigated in detail by Oliver (1972a, b). Crosses of English and French populations of *Pararge megera* (L.) (Lepidoptera, Satyridae) (Oliver, 1972a) gave low mortality in both control and F<sub>1</sub> broods, with no evidence of heterosis, but the various back-cross broods showed greatly increased mortality. There was no disturbance of sex ratio. These populations are believed to have been isolated from one another since the opening of the English Channel, about 7000 years ago. Oliver (1972b) crossed isolated populations of four species of North American Lepidoptera. He found that the degree of incompatibility between populations was proportional to the geographical distance between them. This may reflect evolution since the Pleistocene since the present distribution of North American insects is no older than the post-glacial (Morgan & Morgan, 1980). The results range from total failure of back-cross broods to a level of mortality and heterosis comparable with that shown by the *H. lapponicus* cross. The cross between Pennsylvania and Massachusetts populations of the Nymphalid *Boloria toddi* (Holland) gave levels of heterosis and back-cross mortality similar to those found in the *H. lapponicus* crosses, but no disturbance of the sex ratio. These populations are separated by only about 450 miles, and despite the limited powers of dispersal of this species, there is no reason to suppose that they have been totally isolated from one another since the Pleistocene.

The Spanish and Swedish populations of *H. lapponicus* thus show a degree of genetic difference broadly comparable with that shown by butterflies from areas with only a moderate geographical distance between them. Such differences are associated with the adaptation of each population to its own local environment, and do not reflect steady divergence with time. Oliver (1972b) points out that since any local population is most likely to differentiate from its least isolated neighbouring population, rather than one that is more closely adapted to parallel environmental conditions but is more isolated, the amount of genetic differentiation between two populations should be proportional to the absolute amount of environmental change that has occurred over the distance between them rather than the corresponding net amount of change. This reasoning



envisages a species spreading over an area whose range of environments at the time of colonization was more or less the same as that existing at present. If the changes of environments through time as a result of Pleistocene climatic fluctuations are considered, then isolated populations might be expected to become progressively more different from one another as each became adapted to new conditions—for even though faunal movement has been shown to be the most conspicuous result of climatic change, there must have been changes in the environments inhabited by the various populations. The reason why cumulative changes in response to new conditions have not resulted in local populations having adapted and readapted to the point of speciation is that the scale of faunal movements is such that populations have not remained isolated from one another, but have tended to merge and then become reisolated as areas of suitable environment have coalesced and fragmented. A failure to reach the point of speciation after 10 000 years (and hence 10 000 generations) in isolation implies a degree of evolutionary stability sufficient for isolated populations to remain compatible when they meet again as a result of faunal movements. This is entirely in keeping with the findings of Pleistocene palaeontology.

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