

## CLINES IN THE COLOUR POLYMORPHISM OF *PHILAENUS SPUMARIUS* IN EASTERN CENTRAL EUROPE

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### SUMMARY

Populations of *Philaenus spumarius* (Homoptera) have been investigated in the Baltic States of the USSR, in Czechoslovakia and in Hungary. Colour morph frequencies in these highly polymorphic populations are compared with those reported earlier from northern Italy.

Gradual changes in allele frequencies (clines) transect the study area, which lies in the most central part of the range of *Philaenus* in Europe. From north to south the allele  $p^M$  increases, but  $p^O$  decreases, in frequency. In their distribution, some of the other alleles also showed regional gradients.

The possibility that visual selection varies regionally is dismissed on indirect grounds. Gradients in climatic severity, or in other environmental variables changing concomitantly with climate, are regarded as the primary cause of the observed clines and other differences between regions.

### 1. INTRODUCTION

THE colour polymorphism of the meadow spittlebug, *Philaenus spumarius* (L.) (Homoptera), is determined by combinations of at least seven allelic genes of the  $p$  (for "pigmentation") locus plus a number of non-allelic modifier genes (Halkka *et al.*, 1975). In Europe, except in the northernmost parts and in some isolates, the various genotypes are expressed as more than 10 distinct phenotypes. In many respects, the polymorphism of this insect offers a useful parallel for comparison with the colour polymorphisms in the equally widely distributed but more extensively investigated snails of the helioid group. In the snails, notably in *Cepaea*, the relative importance of various types of selection is now being discussed (*e.g.* Jones, 1973).

In *Philaenus* regional differences in climatic or other non-visual types of selection are manifest as clines hundreds of kilometres in extent, or as area effects of more limited dimensions (Halkka, 1964; Halkka, Raatikainen and Halkka, 1974). But all the work on regional differences accomplished hitherto has been with the populations of northern Europe. The only more southern populations studied critically with regard to regional distribution of colour morphs are those investigated by Raatikainen (1971) in northern Italy.

The present study is an effort to fill in the wide gap between the populations of northern Europe and Italy, and deals with populations from the Baltic States of the USSR, from Czechoslovakia, and from Hungary. One particular topic studied is the possibility that there are clines transecting the European centre of the range of the species. Hitherto, clines have been known only from the periphery of the range.

## 2. COLLECTING

From the Baltic States, 11 samples were collected from Estonia, 4 from Latvia and 4 from Lithuania. The 5 samples from Czechoslovakia come from the eastern half of that country, and the 2 samples from Hungary from the western part of the central plain. Most of the samples are from rather open meadow habitats, and almost all are from quite low altitudes.

The Hungarian samples were collected in June 1974, the Czechoslovakian ones in July 1967, and the Baltic ones in July-August 1966. The *Philaena* populations are temporally very stable (Halkka, 1964), and it appears safe to compare samples collected in different years.

## 3. RESULTS

In Tables 1 and 2, the populations are listed with the northern-most ones at the top. Males and females are presented separately, because many of the colour alleles are not (normally) expressed in the males (Halkka *et al.* 1975).

TABLE 1

Percentages of the six colour morphs among the males. The numbers after the name of each population denote latitude and longitude (e.g.: Toila, 59°30'N, 27°40'E). Total number of males examined at right

	<i>typ</i>	<i>tri</i>	<i>fla</i>	<i>lop</i>	<i>pra</i>	<i>vit</i>	Total
<i>Estonian SSR</i>							
Toila 5930—2740	94.6	0.9	0.9	2.7	—	0.9	443
Vaida 5925—2500	97.7	1.7	—	0.4	—	0.2	539
Haapsalu 5850—2330	90.6	0.8	6.5	1.7	0.4	—	526
Matsalu 5845—2345	81.6	1.9	3.9	10.2	2.2	0.2	411
Vändra 5840—2300	96.9	1.8	0.7	0.4	—	0.2	548
Pöltsamaa 5840—2550	94.6	2.7	0.6	1.7	—	—	519
Soonda 5835—2320	82.4	2.9	—	14.7	—	—	272
Soe 5825—2240	86.6	6.0	—	7.2	0.2	—	515
Pärnu 5820—2445	88.0	4.2	—	5.8	1.0	1.0	191
Mikitamäe 5800—2725	87.4	5.3	1.1	5.5	0.7	—	454
Hargla 5735—2635	89.7	3.2	0.8	6.3	—	—	253
<i>Latvian SSR</i>							
Valmiera 5735—2525	93.8	3.0	0.8	2.2	—	—	506
Saulkrasti 5725—2430	91.8	1.9	3.9	1.2	0.8	0.4	484
Baldone 5650—2415	79.0	6.6	6.6	6.6	0.4	0.8	228
Kalneciems 5640—2540	88.2	3.0	0.6	7.0	0.6	0.6	498
<i>Lithuanian SSR</i>							
Birzai 5615—2435	86.7	3.7	5.7	2.5	1.2	0.2	593
Telsai 5600—2220	90.5	3.2	1.4	4.1	0.3	0.5	587
Kapsukas 5440—2520	86.7	2.0	4.9	2.2	1.6	1.8	492
Punia 5435—2400	88.1	3.8	6.9	9.4	0.5	0.7	605
<i>Czechoslovakia</i>							
Ruzomberok 4900—1915	68.8	5.1	6.7	18.2	—	—	253
Straznice 4855—1720	88.0	2.3	—	4.4	—	5.3	341
Roznava 4840—2030	88.6	1.0	2.8	4.2	0.3	3.1	286
Caradice 4830—1845	95.1	—	—	1.0	—	3.9	203
Smolenice 4825—1730	87.9	2.3	—	5.2	—	4.6	518
<i>Hungary</i>							
Szigliget 4655—1735	76.4	1.8	—	3.6	—	18.2	55
Nagykanizsa 4630—1700	91.3	—	—	4.3	—	4.3	69

The hierarchy of the alleles is rather complex. Roughly, the order of dominance, in the female sex, is as follows:  $p^T$  (*tri*) —  $p^M$  (*mar*) —  $p^L$  (*lat*) =  $p^F$  (*fla*) =  $p^C$  (*lce*) —  $p^O$  (*lop*) —  $p^t$  (*typ*). In this scheme, — denotes dominance, and = co-dominance.

The frequency of each phenotype or group of phenotypes representing a specific allele is expressed as a percentage of the male or female sample size. In every table the sample size is given on the right.

TABLE 2

Percentages of the nine colour morphs among the females. Total number of females examined at right

	<i>typ</i>	<i>tri</i>	<i>lat</i>	<i>mar</i>	<i>fla</i>	<i>lce</i>	<i>lop</i>	<i>pra</i>	<i>vit</i>	Total
<i>Estonian SSR</i>										
Toila	70.6	0.2	3.1	0.2	4.6	3.9	17.2	0.2	—	458
Vaida	82.5	1.8	0.6	0.6	4.6	1.4	8.5	—	—	495
Haapsalu	79.0	0.2	3.1	1.0	7.3	1.1	8.4	—	—	524
Matsalu	71.7	1.3	4.1	1.0	4.1	1.5	14.0	2.0	0.3	392
Vändra	76.6	1.9	1.7	0.2	10.3	1.4	7.9	—	—	582
Põltsamaa	79.0	1.8	1.1	0.7	6.3	2.0	8.9	0.2	—	447
Soonda	75.2	2.6	0.7	—	8.9	0.7	11.9	—	—	270
Soe	72.5	6.4	0.4	2.1	9.5	2.9	5.8	0.4	—	485
Pärnu	70.6	2.7	0.3	1.2	6.4	1.2	16.4	0.9	0.3	329
Mikitamäe	70.2	6.2	0.4	1.8	10.5	1.9	7.8	1.0	—	514
Hargla	72.2	3.2	0.6	1.3	10.4	2.2	9.8	0.3	—	317
<i>Latvian SSR</i>										
Valmiera	73.8	3.4	0.2	0.6	9.6	2.1	9.9	0.4	—	477
Saulkrasti	76.9	2.3	0.9	0.2	9.9	1.8	7.6	0.4	—	567
Baldone	64.0	5.1	—	2.2	17.6	3.7	7.4	—	—	136
Kalnciems	68.2	3.0	0.4	0.2	7.6	2.2	17.1	1.3	—	463
<i>Lithuanian SSR</i>										
Birzai	67.0	4.3	1.2	0.7	14.5	0.7	10.7	0.7	0.2	422
Telsai	70.7	3.5	0.2	0.4	12.7	2.9	8.1	1.3	0.2	520
Kapsukas	68.1	3.4	0.2	2.6	10.9	5.4	7.6	1.0	0.8	503
Punia	51.9	2.6	1.4	3.2	19.0	5.4	16.3	—	0.2	496
<i>Czechoslovakia</i>										
Ruzomberok	55.9	6.8	1.1	2.3	9.0	11.9	9.6	2.8	0.6	177
Straznice	82.3	3.1	—	4.2	3.4	0.8	3.1	—	3.1	262
Roznava	74.6	3.1	—	1.6	8.6	4.7	2.0	2.7	2.7	256
Caradice	81.8	2.8	—	7.0	4.2	2.1	1.4	—	0.7	143
Smolenice	81.7	2.8	1.5	5.8	2.6	0.4	2.6	0.4	2.2	462
<i>Hungary</i>										
Szigliget	81.6	—	—	10.5	—	—	5.3	—	2.6	38
Nagykanizsa	81.4	5.1	—	1.7	3.4	—	3.4	—	5.1	59

In the following discussion, the populations from Estonia, Latvia and Lithuania will be designated "the Baltic group", or "B", and the populations from Czechoslovakia and Hungary will be dealt with together as "CZ-H". Many of the colour morphs show distinctly dissimilar frequencies in B and CZ-H. The phenotype *mar* (mainly genotype  $p^M/p^t$ ; see Halkka, Halkka, Raatikainen and Hovinen, 1973) is much commoner in CZ-H (4.4 per cent) than in B (1.2 per cent). Farther southwest, in Italy, the frequency of *mar* is as high as 10.1 per cent (Raatikainen, 1971). These differences indicate that the frequency of the  $p^M$  allele increases southwards,

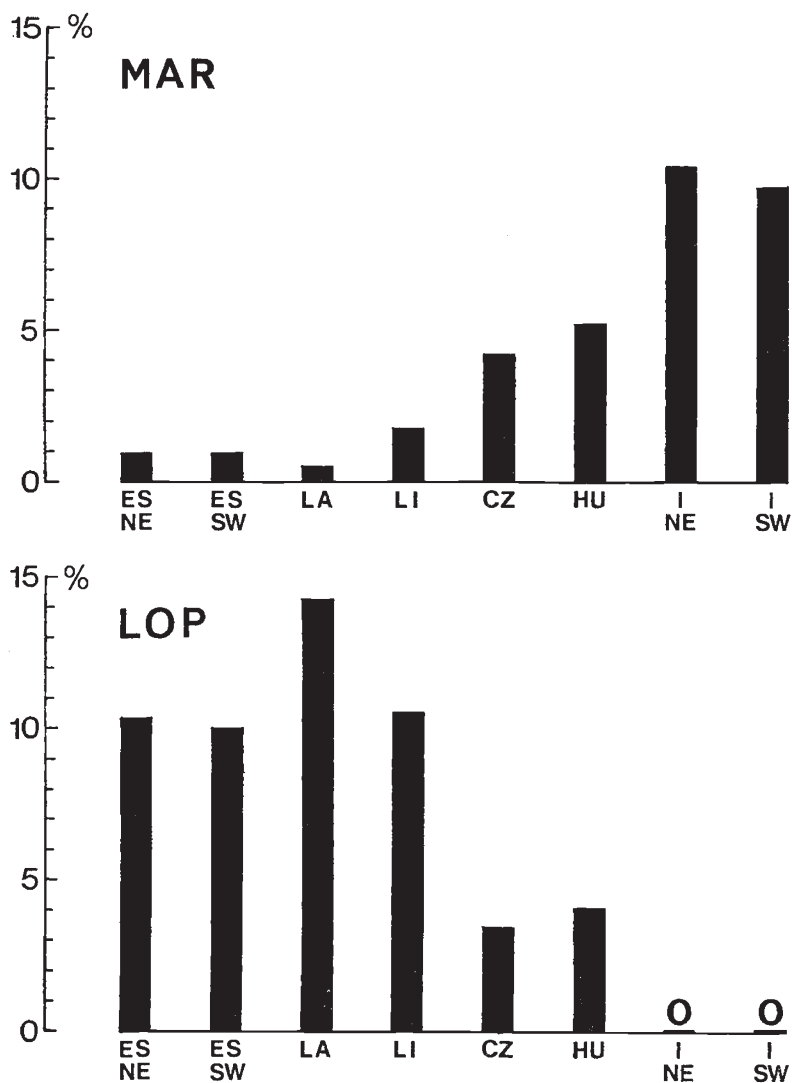


FIG. 1.—Phenotype frequency histograms for females from eight geographical groups of samples. From left to right: NE and SW Estonia, Latvia, Lithuania, Czechoslovakia, Hungary, and NE and SW parts of northern Italy.

possibly as a continuous cline (Fig. 1). The phenotype *mar* can, in fact, result from interaction of  $p^L$  with either  $p^F$  or  $p^C$ , as well as from  $p^M$  (table 3). But in Central Europe the frequencies of the  $p^L/p^F$  and  $p^L/p^C$  heterozygotes are negligible compared with the pooled frequency of the  $p^M$  heterozygotes. This is true because  $p^L$  is very rare in all the three regions, B, CZ-H and Italy (see below).

A cline in the reverse direction appears probable from the frequencies of the allele  $p^O$ . The *lop* group of phenotypes determined by this allele has an average frequency of 10.2 per cent, in the 20 populations of B, but only of 3.4 per cent in the 7 populations of CZ-H. In the samples collected by

Raatikainen (1971) from northern Italy, this group comprises only 1.4 per cent of the (female) individuals. Again, B and Italy are very dissimilar in allele frequency, and CZ-H is intermediate.

In the dominance hierarchy, the allele  $p^O$  is close to the bottom (table 3). The only allele recessive to it,  $p^t$ , is about equally common in B, CZ-H and Italy (see below). The cline in the frequency of  $lop$  (fig. 1) must therefore reflect a cline in the frequency of  $p^O$ .

No such pronounced regional differences are manifested by the other five established alleles,  $p^T$ ,  $p^L$ ,  $p^F$ ,  $p^C$  and  $p^t$ .  $p^T$  is the top dominant allele in the dominance hierarchy series in both sexes and therefore always detectable by the presence of the appropriate phenotype, *tri*. This phenotype is rather low

TABLE 3

*The relevant allele combinations and the corresponding phenotypes (in the female sex)*

	$p^T$	$p^M$	$p^L$	$p^F$	$p^C$	$p^O$	$p^t$
$p^M$	<i>tri</i>	<i>mar</i>	<i>mar</i>	<i>mar</i>	<i>mar</i>	<i>mar</i>	<i>mar</i>
$p^L$	<i>tri</i>	<i>mar</i>	<i>lat</i>	<i>mar</i>	<i>mar</i>	<i>lat</i>	<i>lat</i>
$p^O$	<i>tri</i>	<i>mar</i>	<i>lat</i>	<i>fla</i>	<i>lce</i>	<i>lop</i>	<i>lop</i>

in frequency in B (3.0 per cent), as well as in CZ-H (2.7 per cent). Nor is it common in Italy (1.6 per cent; Raatikainen, 1971). As almost all the *tri* individuals are heterozygotes, the average frequencies of the  $p^T$  allele in the three regions mentioned are about 0.015, 0.014 and 0.008, respectively.

The frequency of colour morph *lat* (allele  $p^L$ ) in B is 1.1 per cent, in CZ-H 0.6 per cent, and in Italy (Raatikainen, 1971) 2.0 per cent. The allele  $p^L$ , although rare in B, was found in 18 of the 20 populations from this region. This allele is only exceptionally expressed in the males, and the frequencies have all been calculated for the female sex only.

The phenotype *fla* (allele  $p^F$ , or  $p^C$ ) is more frequent than either *tri* or *lat* in both B and CZ-H (table 1). A point worth noting although not indicated in the table, is that the majority of the *fla* individuals in CZ-H belong to the *fla-2* phenotype described by Raatikainen (1971). Previously, this phenotype was known only from Italy.

The phenotypes *gib* and *lce* (allele  $p^C$ ) are about equally frequent in B (2.2 per cent) and CZ-H (2.9 per cent). In northern Italy, Raatikainen (1971) found only one *gib* and no *lce* among 2116 females.

The phenotypes *vit* and *pra* are rather rare in both B and CZ-H, and also in northern Italy (Raatikainen, 1971). The genetic basis of these colour morphs is unknown, but they may well be determined by specific alleles, " $p^V$ " and " $p^P$ ". If these alleles really exist, the number of alleles at the  $p$  locus in the central European populations is (at least) 9.

The assembly of phenotypes grouped under the denomination *typ*, although the last to be considered, is not least in importance. In the females, the allele determining this group of phenotypes,  $p^t$ , is the bottom recessive. All the *typ* females are thus  $p^t/p^t$ , which means that  $(p^t)^2$  frequencies can be read directly from table 1. Within the *typ* group, phenotypic variability is much more extensive in the south than in the north of Europe. Taking the group as a whole, no great difference with regard to its frequency exists between B (72.8 per cent) and CZ-H (77.0 per cent). In Italy, according to Raatikainen (1971), the corresponding frequency is 77.8 per cent. As shown by these values, the average frequency of the allele  $p^t$  exceeds 0.8 in all three

regions. A typical feature of the colour polymorphism of *Philaenus* is that much of the geographical variability in allele frequencies occurs as a "fine adjustment" of the relative frequencies of the rare alleles. Although these 6 (or perhaps 8, see above) alleles *together* comprise only about 0.2 of the total, their frequencies may be very sensitive indicators of variation in the external environment.

#### 4. DISCUSSION

The mean temperature of July is about 17°C in the Baltic States, 21°C in Hungary, and the same in northern Italy. Between B and CZ-H, there is no great difference in the mean temperature of January, but Italy (about 0°C) differs from Hungary (-2°C). These differences in climate between the three regions may well affect the various colour morphs dissimilarly. In northern Europe, the colour phenotypes have been shown to differ in their "north tolerance", *i.e.* their limits of distribution do not coincide and in some cases differ widely (Halkka, Raatikainen and Halkka, 1974).

The populations of Northern Europe (Sweden and Finland) are separated from those of the Baltic States by the Baltic Sea. This sea appears to constitute an effective barrier to the migration of many animals, including *Philaenus*. The populations of southern Finland and northern Estonia, although only about 50 km apart, show profound differences in allele frequencies (Halkka *et al.*, 1973). Thus, either a cline or an abrupt transition in gene frequencies exists in the chain of populations (about 400 km long) occupying the terrain bordering the Gulf of Finland between Toila (table 1) and southeastern Finland. In this chain the morphs *pra* and *vit*, which are present in Estonia but absent from continental Finland, reach their northern limits. The dropping out of these, and the changes in the frequencies of the other morphs, may be due to transitions in climate or in some other factors that vary concomitantly with climate.

In polyphagous herbivores like *Philaenus* or *Cepaea*, climate may influence allele frequencies either directly or *via* regional peculiarities in the distribution of the most important food-plant species. In *Philaenus*, the colour morphs differ in their choice of food-plants (Halkka, in preparation). In studies on the selective mechanisms, an experimental approach or direct field observation (for *Cepaea*, Richardson, 1974) is usually necessary before a decision can be made on whether climatic variables act directly or indirectly.

Some of the apparent differences of opinion about the effects of climatic selection, frequent in the literature on *Cepaea* (*e.g.* Guerrucci-Henrion 1966; Harvey, 1971*a, b*) may be due to the complexity of the indirect actions of such selection. Nothing has been reported about whether the colour morphs of *Cepaea* differ in their choice of food-plants.

Some of the principles governing climatic selection in *Cepaea* were proposed in the fifties (Lamotte, 1959), and more recent work is concerned with clarifying the relative significance of visual and non-visual selection. Arnold (1971) and others have presented evidence that in areas with reduced visual selection the impact of climatic selection is pronounced. Regional differences in the intensity of predation, as observed in *Cepaea*, have not been reported for *Philaenus*. In the spittlebug, visual selection has been claimed to operate by the apostatic principle (Owen and Wiegert, 1962), or by the principle of warning coloration (Thompson, 1973). Neither hypothesis has

been corroborated by experiments or observations in the field. A comprehensive search of the literature on food lists of well over 100 species of European birds is now in progress, but as yet has failed to disclose any effective predator of *Philaenus* (Halkka and Kohila, in preparation). So far, there are no grounds for expecting visual selection to discriminate in any way between the apparently non-cryptic black-and-white phenotypes (*mar*, *lat* and *lce*, for instance).

In conclusion, we suggest that gradients of climatic severity (or some concomitant) along the transect from Italy (about 44 N) to Estonia (about 59 N) account for the clines and other regional differences in the frequencies of *p* alleles in *Philaenus*. The suggested clines cross the centre of the range of this species in Europe.

There is very little overlap between the geographical regions where studies have been made on morph or gene frequencies in natural populations of *Cepaea* and *Philaenus*. It would perhaps be rewarding to make studies on *Philaenus* in the localities used in investigations on the ecological genetics of snails. Ecologically snails and spittlebugs are probably largely independent of each other, and it would be of interest to examine whether there is any parallelism in clines or area effects.

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