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The Colour/Pattern Polymorphism of Philaenus spumarius (L.) (Homoptera: Cercopidae) in England and Wales

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The colour/pattern polymorphism of *Philaenus spumarius* (L.) (Homoptera: Cercopidae) in England and Wales

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CONTENTS

SUMMARY

The spittlebug *Philaenus spumarius* is a common and widespread Palaearctic insect exhibiting a striking dorsal colour/pattern polymorphism, which is conveniently divisible into eight 'melanic' and five 'non-melanic' phenotypes. The polymorphism is controlled by seven alleles at a single autosomal locus with complete or partial female-limitation and variable penetrance in males of certain melanic phenotypes. It is a universal polymorphism but, despite much investigation, little is known of the factors which maintain it or influence morph frequency. Patterns of geographic variation are useful in both these contexts.

This study presents an extensive survey of morph frequency variation in 548 populations of *P. spumarius* across England and Wales. Frequencies of individual phenotypes within the melanic category vary heterogeneously; frequency variation for a 'core' group of five phenotypes is broadly in parallel, whereas the other three vary independently. Furthermore, the degree of penetrance in males varies between the melanic phenotypes, which in turn varies between populations; one consequence is that higher melanic frequencies are associated with enhanced expression of certain melanic phenotypes in males. Comparisons are made with previously reported populations around a point source of serious gaseous and particulate pollution in the Cynon Valley, South Wales and with intensively studied populations in southern Finland.

The data indicate a pronounced 'urban effect' on morph frequencies, with populations in major conurbations exhibiting higher mean melanic frequencies ($\bar{x} = 19.1\,\%$) than rural ones ($\bar{x} = 7.3\,\%$).

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Populations around three (of the eight investigated) point sources of atmospheric pollution show elevated melanic frequencies, although all are substantially below those recorded from the Cynon Valley. Although Finnish populations exhibit substantially higher melanic frequencies than analogous rural populations in Britain, the strict female-limitation of certain melanic phenotypes in Finland is relaxed in British populations, where male melanics are approximately 20-fold more frequent.

The universality and stability of this polymorphism strongly suggest the overriding influence of natural selection in its maintenance. The predominance of the phenotypes GIB and LCE (mostly black, but with pale heads) over the completely black form (LOP) in populations with the highest overall melanic frequencies demonstrates that the selective agent(s) favouring melanics in intensely polluted areas cannot be operating simply for melanism *per se*. The near-equality of melanic frequencies between the sexes in the majority of populations reported here suggests that the model of genetic control established for populations in the Cynon Valley also applies to most British populations and remains distinct from the model applying to Finnish populations.

1. INTRODUCTION

Polymorphic variation in animal colour and pattern, especially in invertebrates, is significant because it is thought to have important effects on phenotype fitness. A number of visible polymorphisms have therefore attracted considerable attention in ecological genetics over several decades. Good examples are shell colour and banding in pulmonate snails of the genus *Cepaea* (Jones *et al*. 1977), melanism in moths (notably *Biston betularia*; Berry 1990) and the ladybird *Adalia bipunctata* (Majerus 1994) as well as colour/pattern in the spider *Enoplognatha ovata* (Oxford 1985).

Studies of geographic variation in morph frequencies, and its interpretation in terms of spatial changes in the environment, retain a classic role in ecological genetics by indicating possible selective agents (Endler 1977, 1986). However, other deterministic influences, particularly gene flow, and stochastic processes such as genetic drift and the founder effect may complicate the interpretation of inter-population differences in morph frequencies (Oxford 1989, 1991; Brakefield 1990).

The colour/pattern polymorphism in the common spittlebug *Philaenus spumarius* (L.) has received detailed attention on all three continents covered by its extensive Palaearctic range. Much of this interest owes its inspiration to the pioneering work of O. Halkka and his colleagues working in Finland and neighbouring countries (Halkka & Halkka 1990). Hitherto however, the species has received little attention in Britain; this paper reports on an extensive survey of the polymorphism in England and Wales. Elsewhere (A. Stewart & D. Lees, unpublished data) we attempt to explain the observed patterns of geographic variation in terms of environmental factors.

2. THE SPECIES AND ITS COLOUR/PATTERN POLYMORPHISM

(*a*) *Ecology and geographic range*

The spittlebug *Philaenus spumarius* is one of the commonest phytophagous insects in temperate regions, where it occurs in most terrestrial habitats but most frequently in meadows, waste ground and roadsides. Population densities vary widely, but can reach the order of hundreds per m^2 with nymphal densities significantly in excess of $1000\ m^{-2}$ occasionally recorded (Wiegert 1964; Zajac & Wilson 1984). It is highly polyphagous, with more documented host species than any other herbivorous insect (Weaver & King 1954), a striking feature of which is its preference for nitrogen-fixing plants (Thompson 1994). Nymphs of the family Cercopidae, together with the Aphrophoridae, are unique in producing a frothy secretion that forms a globular spittle mass around the body, the function of which is thought to be related to protection either from natural enemies or desiccation (Whittaker 1970). The species is known to be univoltine across most of its range, although Drosopoulos and Asche (1991) have suggested that it may be at least partly bivoltine in Greece at elevations below 1000 m. In Britain, *P. spumarius* hatches in May, undergoes five nymphal instars (all monomorphic pale green or yellow) and emerges as an adult in late June to early July. Adults are present at least until October, although males generally do not survive as long as females.

The distribution of *P. spumarius* covers much of the Holarctic region, encompassing several major climatic and vegetation zones. In Europe, it extends from north Finland to the Mediterranean. It has been reported from north Africa, most provinces of the former Soviet Union (Beregovoi 1972; Whittaker 1972) as well as China and Japan (Nast 1972). It is believed to have been introduced into North America, where it now occupies a split distribution in two broad bands down the eastern and western seaboards of the U.S.A. and Canada (Thompson 1984). In addition, it has recently colonized New Zealand (Archibald *et al*. 1979) and the Azores (Quartau *et al*. 1992). It also became established at high elevation on the island of Hawaii in the 1940s (Anon 1945; W. J. Booth, personal communication).

(*b*) *Phenotypic variation*

Adult *P. spumarius* exhibit considerable colour and pattern variation on both the dorsal and ventral body surfaces. Certain parts of the ventral surface, particularly the abdomen and frontoclypeus (face), vary in pigmentation from pale yellow-brown to black and some previous workers have attempted to recognize distinct colour morphs (Beregovoy 1970; Svala & Halkka 1974; Thompson 1988). However, the nature

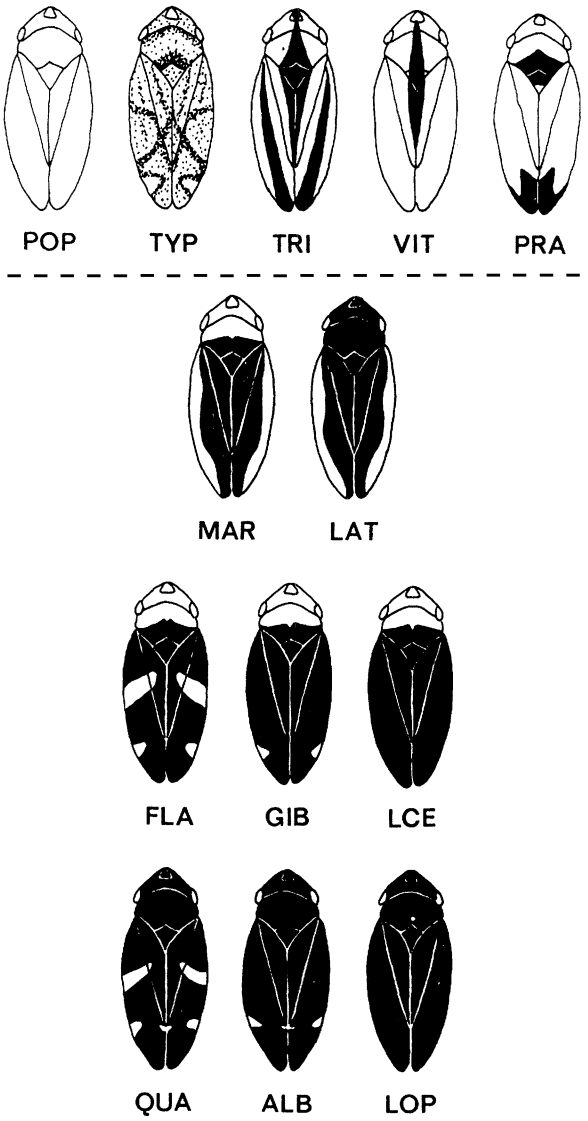

Figure 1. The thirteen principal colour/pattern phenotypes in *Philaenus spumarius* referred to in this paper. Non-melanic phenotypes above the dashed line, melanics below. Key: POP, *populi*; TYP, *typicus*; TRI, *trilineatus*; VIT, *vittatus*; PRA, *praeustus*; MAR, *marginellus*; LAT, *lateralis*; FLA, *flavicollis*; GIB, *gibbus*; LCE, *leucocephalus*; QUA, *quadrimaculatus*; ALB, *albomaculatus*; LOP, *leucophthalmus*.

of ventral variation and how it relates to dorsal variation is still to be established firmly (West 1990; West & Lees 1996).

By contrast, the colour/pattern variation on the dorsal surface constitutes a very striking polymorphism which has been described and illustrated by various authors (Halkka 1962; Farish 1972; Halkka *et al.* 1973; Lees *et al.* 1983). Some have chosen to distinguish a large number of colour morphs (Hamilton 1982) or emphasised the existence of intermediate forms (Harper 1974). However, it is now generally agreed that there are 13 colour phenotypes into which most individuals from most populations can be categorized. These phenotypes have been ascribed varietal names which are conveniently referred to by three-letter abbreviations, as follows: *populi* (POP), *typicus* (TYP), *trilineatus* (TRI), *vittatus* (VIT), *praeustus* (PRA), *marginellus* (MAR), *lateralis* (LAT), *flavicollis* (FLA), *gibbus* (GIB), *leucocephalus* (LCE), *quadrimaculatus* (QUA), *albomaculatus* (ALB), *leucophthalmus* (LOP) (see figure 1). The VIT and PRA morphs are often considered to be minor modifications of TRI; however, we have chosen to present the data for these morphs separately. Lees *et al.* (1983) have drawn a useful distinction between those morphs which are essentially pale with limited dark patterning (POP, TYP, TRI, VIT, PRA) and the remainder which are dark with various combinations of pale markings on the wings, head and prothorax. The two groups will be referred to as the non-melanics and melanics respectively.

(*c*) *Genetic control*

The genetic control of the polymorphism is complex (Halkka *et al.* 1973). Seven alleles at a single autosomal locus have been identified which code for the various colour/pattern phenotypes as follows: P^T, TRI + VIT; P^M, MAR; P^L, LAT; P^F, FLA-F; P^C, FLA-C + GIB + LCE; P^O, QUA + ALB + LOP; P^t, TYP + POP. The genetic distinction between the phenotypes within the 'C' and the 'O' groupings, between POP and TYP and between TRI and VIT are not clear but may well be controlled by modifier alleles at one or more separate loci, possibly linked to the main colour locus. The genetic basis of PRA is unknown. The various phenotypes can be arranged in a dominance hierarchy with complex patterns of dominance and co-dominance between certain morphs. Halkka *et al.* (1973) suggested that this dominance hierarchy differs between the sexes (see figure 2), although we have questioned this interpretation of their results (Stewart & Lees 1988). Nevertheless, omitting the co-dominance formed between FLA and certain other melanics, the dominace hierarchy in females (and most probably in males as well) can be represented as:

$$\text{TRI} > \left\{\begin{matrix}\text{MAR}\\ \text{FLA-F}\end{matrix}\right\} > \text{LAT} = \text{'C'-group} > \text{'O'-group} > \text{TYP}.$$

(*d*) *Phenotypic expression*

Two aspects of this polymorphism are of particular interest. The first concerns its interaction with sex. The frequency of individual morphs is rarely equal in the two sexes. Differences are particularly marked in the melanic morphs, which are generally rarer in males than in females. The effect is more pronounced in certain melanic phenotypes, such that in many Finnish populations MAR, LAT, GIB and LCE are entirely confined to the females. However, this generalization does not apply to all populations. In certain high altitude (Halkka *et al.* 1980) or high latitude populations in eastern Europe (Halkka *et al.* 1974*b*) and many British populations (this paper), the female limitation is relaxed and the same morphs show greatly increased or complete penetrance in males.

The second complication concerns the variable expression of the melanic genes in males, which is never as clear as in females. In male MAR and LAT for example, the pale wing border may be quite indistinct,

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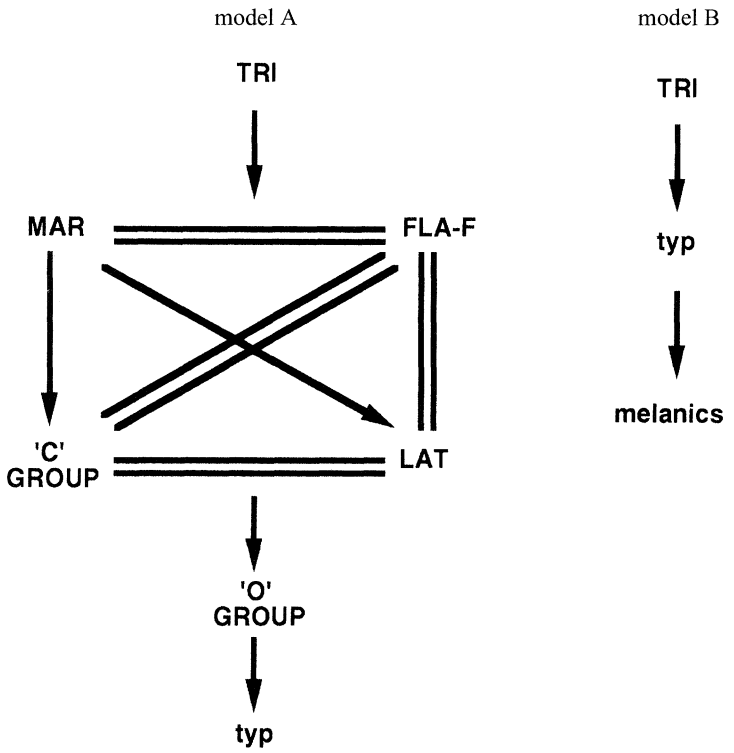

Figure 2. Models of dominance hierarchy for the principal phenotypes at the major colour locus in *Philaenus spumarius*. Model A depicts the hierarchy for females only in Finnish populations (Halkka *et al.* 1973), but both sexes in British populations (Stewart & Lees 1988; D. Lees, J. West & A. Stewart, unpublished data). Model B applies to males only in Finnish populations (Halkka *et al.* 1973). Arrows indicate direction of dominance; double lines indicate co-dominance.

even though careful crossing experiments have confirmed their genetic status (for illustrations and details of crosses see Stewart & Lees 1988). The clarity of expression of these melanic alleles also varies geographically; the clearest expression is generally to be found in populations that have the highest overall melanic frequencies (Lees & Dent 1983; Lees & Stewart 1987), where it may be assumed that selection for melanism and the clarity of expression of melanic phenotypes is strongest.

3. PREVIOUS STUDIES OF GEOGRAPHIC VARIATION IN MORPH FREQUENCIES

An extensive literature exists on geographic variation in *P. spumarius* morph frequencies across the species' range. Halkka and colleagues have recorded large-scale variation in morph frequencies in Scandinavia (Halkka *et al.* 1967*b*; 1974*b*; Boucelham & Raatikainen 1987) and in eastern central Europe, across the Baltic states of the former U.S.S.R., former Czechoslovakia and Hungary (Halkka *et al.* 1975*d*, 1976*b*). Within the Palaearctic region, other studies have reported morph frequencies in the U.S.S.R. (Beregovoi 1966, 1972; Whittaker 1972) and former Czechoslovakia (Honek 1984). However, with the exception of a detailed survey in northern Italy (Raatikainen 1971) and a few isolated samples from France and Germany (Halkka *et al.* 1967*b*), populations in western parts of continental Europe have received little attention. Morph frequency variation in north American populations has been widely reported (Owen & Wiegert 1962; Farish & Scudder 1967; Thompson & Halkka 1973; Thompson 1984, 1988; Boucelham *et al.* 1988) and the result of a relatively recent introduction into New Zealand has been investigated (Thompson 1983; Lees 1993).

Although the detailed conclusions from these studies are not always complementary, it is now evident that control of variation in morph frequencies in this species operates at a number of spatial levels. At one extreme, several studies have revealed large-scale morph-frequency clines, typically spanning distances of the order of hundreds of kilometres (Halkka *et al.* 1974*b*, 1975*d*, 1976*b*; Thompson 1984, 1988; Boucelham & Raatikainen 1987), which have been interpreted as the product of some sort of climatic selection.

At a more regional level (10–100 km), trends in morph frequencies become more difficult both to identify and to interpret. Altitudinal effects, again presumed to be the result of climatic selection, have been examined but with variable results; Halkka *et al.* (1980) and Berry & Willmer (1986) suggested increases in melanism with altitude, but Boucelham & Raatikainen (1987) and Boucelham *et al.* (1988) could not corroborate this, although there was agreement regarding a negative correlation with TRI frequencies.

At a local level, the influence of habitat type has been investigated (Boucelham & Raatikainen 1984; Halkka *et al.* 1974*a*; Halkka & Halkka 1990). Differences in morph frequency have been demonstrated even between small habitat 'islands' only a few metres apart (Halkka *et al.* 1970, 1976*a*). The most

fine-grained differentiation occurs on different plant species within the same population (Halkka & Mikkola 1977; Halkka *et al.* 1967*a*, Whittaker 1968).

The temporal stability of these patterns (Halkka 1976*a*; Halkka 1978) and the return to polymorphic equilibrium following experimental perturbation of natural populations (Halkka *et al.* 1975*b*) strongly indicate the overriding importance of some sort of natural selection (Endler 1986). However, the influence of random stochastic effects has been suggested in the particular circumstances of small isolated island populations (Brakefield 1990; Halkka *et al.* 1970, 1974*a*), and elegantly demonstrated in island populations which have been subject to periodic catastrophic disturbance (Halkka *et al.* 1975*c*).

4. MORPH FREQUENCY VARIATION IN BRITAIN

Hitherto, geographic variation in *P. spumarius* morph frequencies has received little attention in Britain, other than in studies of a limited number of populations (Hutchinson 1963; Adenuga 1968; Whittaker 1968; Berry 1983). More extensive information was reported by Lees *et al.* (1983) in a pilot survey of 48 populations across England and Wales. Although 31 of these samples were from South Wales, the data provided early indications of several important ways in which the genetic composition of British *P. spumarius* populations differed from previously studied ones in Finland and Scandinavia:

1. Several populations were discovered in which total melanic frequencies were considerably greater than those reported in earlier studies. These populations are all in urban areas, contrasting with frequencies in rural populations which rarely exceeded 5–10%. Based on a limited number of samples, the association between higher melanic frequencies and urban areas is weak but nevertheless significant (Lees *et al.* 1983). A most dramatic demonstration of this effect is found in the Cynon Valley in South Wales, which until 1990 contained a phurnacite smokeless fuel factory which was a significant single source of intense local particulate and gaseous air pollution. Melanic frequencies immediately adjacent to the site of this factory are higher (more than 95%) than anywhere else in the species' entire range (Lees & Dent 1983). These initial indications of urban and pollution influences on the polymorphism invite comparison with other cases of industrial melanism in such species as *Biston betularia*, *Phigalia pilosaria* and *Adalia bipunctata* (reviewed in Lees 1981) and contrast with the findings of Thompson and Halkka (1973) who were unable to detect any sign of an urban/pollution effect on the *P. spumarius* polymorphism in Chicago.

2. There are important differences in the representation of certain morphs in the two sexes. Phenotypes which are strictly female-limited in Finnish populations (MAR, LAT and to a lesser extent GIB, LCE) regularly occur amongst males in British populations, though often at lower frequencies than in the females. Furthermore, the other melanic forms (FLA and the 'O'-group phenotypes) show little sign of any differential representation in the two sexes. Consequently, melanic frequencies tend to be approximately equal between the sexes in many populations; in Finland, melanic frequencies in males rarely exceed 1% and are considerably less than those in females (which are typically around 10%).

3. There is also an indication that the relative contributions of the different morphs to the overall melanic category differ both between the sexes and between populations with contrasting melanic frequencies. In rural populations with low overall melanic frequencies, MAR or LAT are more common than in urban areas, where GIB and LCE tend to predominate. A further complication is apparent in areas of particularly high melanic frequency such as the Cynon Valley, where most melanic females are either GIB or LCE, but the male melanics are phenotypically closest to QUA, ALB or LOP.

5. SURVEY OBJECTIVES

Many of the trends suggested above are only tentative, based on relatively few samples. Against this background, this survey was designed to address the following questions.

1. Is there a general 'urban effect' on melanic frequencies, complementing the earlier finding by Lees & Dent (1983) that *P. spumarius* exhibits industrial melanism?

2. Are there other examples of local adaptation to extreme local air pollution or is the situation in the Cynon Valley unique?

3. Is the characteristic pattern of adaptation found in the Cynon Valley populations (near-equality of melanic frequencies between the sexes, increased penetrance and clearer expression of melanics in males) repeated across Britain, or are there areas containing populations which show greater affinity to the Scandinavian model of the polymorphism?

4. Are there large-scale geographic trends in phenotype frequencies across Britain?

6. METHODS

(*a*) *Sampling*

Samples were collected using standard sweep nets and hand-held aspirators (pooters). The sampling period covered the months of July, August and September. The area searched at a site and the time taken to collect a standard sample varied according to population density, but sampling areas never exceeded 1–2 ha and many urban sites were considerably smaller than this.

P. spumarius is highly polyphagous on a wide variety of herbaceous plant species; it is rarely recorded on grasses or trees. We found that thistles (particularly *Cirsium arvense* L. but also related species) were the most favoured host plants, although many samples were collected from a mixture of plant species. In general, the most productive habitats for sampling were ruderal ones: waste ground, roadside verges, disused industrial workings and other disturbed habitats.

The choice of sampling locations reflects the dual objective of complete geographic coverage of England

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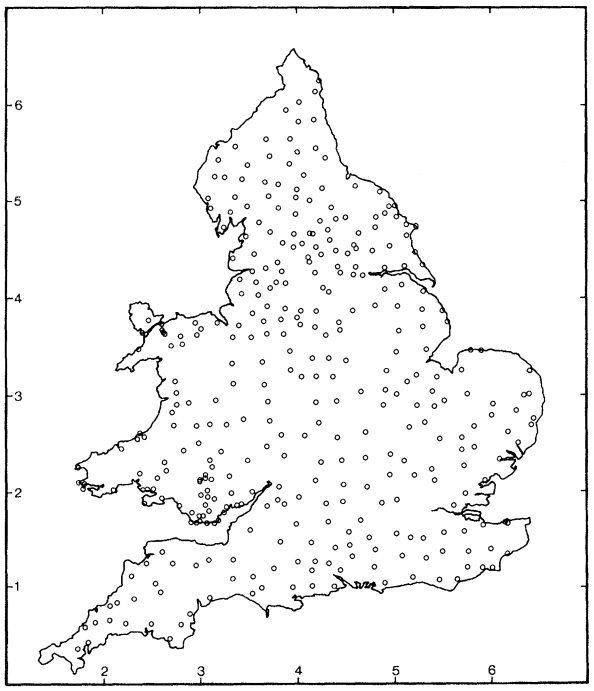

Figure 3. Map of sampling locations for 367 rural populations of *Philaenus spumarius* in England and Wales. Sample sites within major conurbations are omitted, but shown in detail in figure 4. Labelling of axes refers to 100 km intervals on the National Grid.

and Wales combined with testing for an 'urban effect' on morph frequencies; the latter dictated that the principal conurbations received proportionally more sampling effort relative to their area. In rural areas, we attempted wherever possible to collect samples at 20–30 km intervals (see figure 3). Samples in the seven major conurbations were more closely but irregularly spaced, reflecting the availability of suitable habitats (see figure 4).

A broad classification of the habitat type (wet or dry and disturbed or established) was recorded for each site, together with a list of the principal host plants from which *P. spumarius* was collected. Each site was categorized as either urban or rural, based on its position in relation to the approximate outlines of the major conurbations as depicted on 1:50000 Ordnance Survey maps.

(*b*) *Scoring*

In general, attributing female *P. spumarius* to one of the 13 possible phenotypes is straightforward; individuals that are intermediate in some way are very rare (< 1%). The main scoring difficulties occur with males, particularly in the distinction between certain melanic morphs and between melanics and very dark TYP individuals. Amongst the melanics, MAR and LAT are particularly difficult to identify in males, because the pale costal wing border is greatly reduced in width and is often interrupted. The other main difficulty concerns distinguishing between those melanics with and without a pale head. In pale-headed females, the vertex (head) and anterior margin of the prothorax is always clear creamy white. Males however have variable amounts of dark mottling in these positions, which grade into uniform black. The darker of these individuals were originally thought to be attributable to LOP (or the other 'O'-group phenotypes), but detailed crossing experiments have shown that many are genetically LCE (Stewart & Lees 1988).

(*c*) *Data analysis*

Phenotype frequencies were arcsine transformed before analysis. Following Greenwood (1974) and others, an index of polymorphic diversity was calculated using a modification of the Shannon-Weiner formula:

$$H_S = -\sum_{i=1}^{S} p_i (\ln p_i)$$

where p_i is the frequency of the i_{th} phenotype and s is the number of phenotypes represented in the particular population. Diversity values generally range from 0.5 to 2.5. Heterogeneity between the sexes in the penetrance of the melanic phenotypes as a group was tested using chi squared, with d.f. = 1.

The relative complexity of the polymorphism and the large number of populations necessitated the use of multivariate statistical techniques to simplify the data. Principal Components Analysis (PCA) was used to reduce data on the 13 colour morphs to a smaller number of principal components which were linear but orthogonal combinations of the original variables and described the data more succinctly. Discriminant Function Analysis (DFA) was used to separate geographic groups of data. As in PCA, the extracted components, or discriminant functions, were linear combinations of the original percentages, but in this case calculated to produce the maximal discrimination or separation between predetermined groups. PCA and DFA were performed on percentages rather than the original data, in order to remove the effect of sample size. In both cases, the data were standardized (to produce zero means and unit variances) before extraction of the eigenvalues.

7. RESULTS

(*a*) *The dataset*

This paper draws on data from all populations of *P. spumarius* sampled since 1976, including those reported previously (Lees & Dent 1983; Lees *et al.* 1983; Lees & Stewart 1988). The complete dataset comprises 103 893 individuals in 714 samples from 548 populations in England and Wales. The survey was completed between 1983 and 1987. Some populations, particularly in South Wales, were sampled more than once; throughout the following analyses, such repeat samples are combined because there is no indication of heterogeneity between years.

Certain subsets of the data have received separate analysis, because they constitute particularly interesting local patterns of morph frequency variation. These include 36 populations from the highly polluted Cynon Valley in South Wales (Lees & Dent 1983), 21 populations in Cardiff docks (Lees & Stewart 1987) and various populations on the islands of Skomer and

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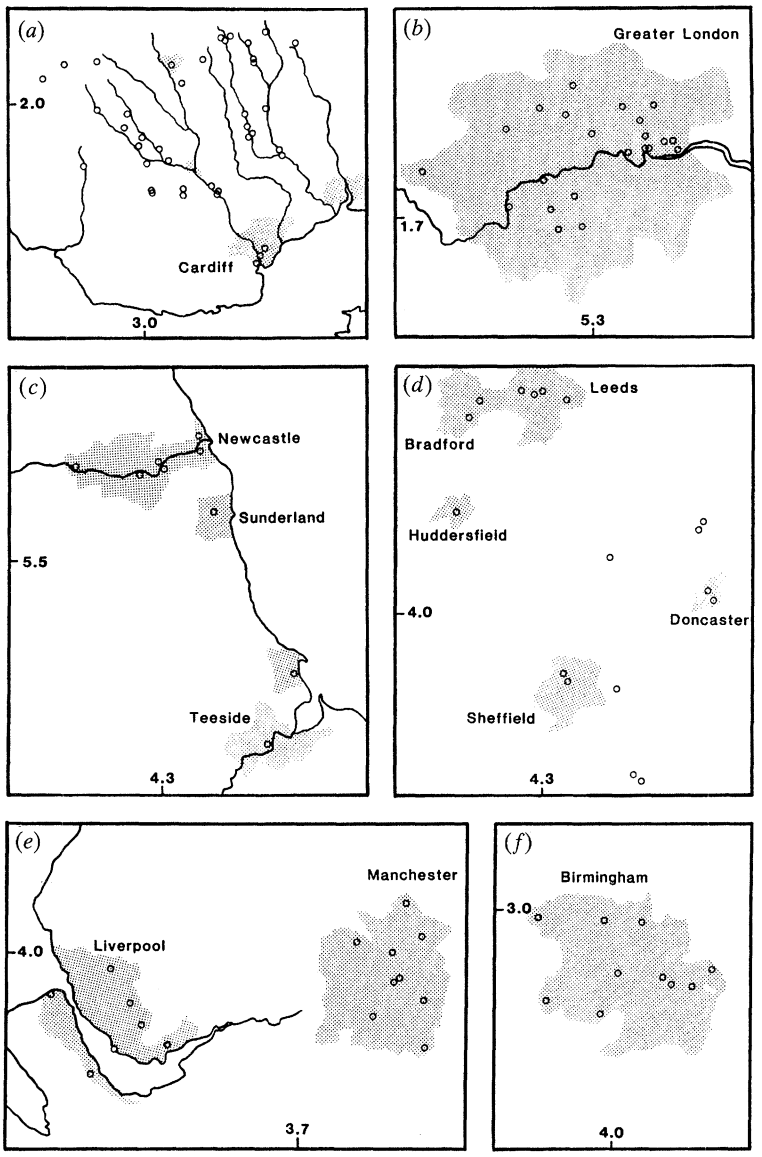

Figure 4. Map of sampling locations for 122 urban populations of *Philaenus spumarius* within 7 major conurbations: (*a*) South Wales, (*b*) London, (*c*) northeast England, (*d*) Industrial northern England, (*e*) Liverpool and Manchester, (*f*) Birmingham. Labelling of axes refers to 100 km intervals on the National Grid. Stippling represents urban areas.

Skokholm off the Pembrokeshire coast (unpublished data). These studies describe relatively fine-grained morph frequency variation in response to particular local environmental conditions at a level which is too detailed spatially for the large-scale treatment sought here. Summary data from these local studies are referred to for comparison, but otherwise these populations have been excluded from this analysis which considers variation among the remaining 489 populations, comprising 77787 individuals from 519 samples.

(*b*) *Overview of morph frequencies*

Summary statistics for each phenotype and for the melanics as a combined category are given in table 1. Some difficulties were experienced in scoring certain very dark TYP individuals, particularly amongst males. We present these as a separate 'dark-TYP' category, but are nevertheless satisfied that they are distinct from any of the melanics; we suggest that they are almost certainly of the TYP genotype, subject to possible genetic or environmental modifier effects. A small number of individuals ($< 0.2\%$) were not readily classifiable into any of the phenotypes and are presented under two 'other' categories according to whether they appeared closest to melanics or non-melanics. Most seemed to be variants of either TYP or LOP and have accordingly been combined with one of these phenotypes in subsequent analyses. For certain analyses and in accordance with their mode of genetic control, GIB+LCE and QUA+ALB+LOP were combined. Likewise, VIT and PRA were assumed to be minor variants of TRI and therefore combined with it. This leaves eight major phenotypic groups. Although POP and TYP are probably controlled by the same allele at the major colour/pattern locus (Halkka *et al.* 1973), POP constitutes a sufficiently large proportion of most populations to merit separate treatment. Approximately 50% of all individuals

Table 1. *Summary data for* Philaenus spumarius *colour morph frequencies in England and Wales*

(Based on 489 populations in England & Wales i.e. excluding 36 populations in the Cynon Valley (Lees & Dent 1983), 21 populations in Cardiff docks (Lees & Stewart 1988) and populations on Skokholm and Skomer (unpublished data).

	phenotypes as scored						phenotypic groups				
	totals		frequency		CPR[a]			frequency		CPR[a]	
	males	females	mean freq.	s.d.	mean	s.d.		mean freq.	s.d.	mean	s.d.
POP	10043	5344	19.92	4.20	1.51	0.35	POP	19.92	4.20	1.51	0.35
TYP	19297	17267	46.35	7.78	0.91	0.09					
dark-TYP	1188	567	2.32	0.90	2.09	1.21	TYP	48.81	7.86	0.94	0.08
other non-melanics	91	39	0.15	0.25	0.67	1.57					
TRI	7053	4156	14.64	4.18	1.43	0.33					
VIT	1323	2361	5.08	3.00	0.42	0.17	TRI	20.43	5.94	1.00	0.14
PRA	160	277	0.62	0.74	0.42	0.57					
MAR	39	430	0.63	0.38	0.06	0.09	MAR	0.63	0.38	0.06	0.09
LAT	157	922	1.43	0.69	0.13	0.09	LAT	1.43	0.69	0.13	0.09
FLA	294	682	1.19	1.09	1.19	2.34	FLA	1.19	1.09	1.19	2.34
GIB	298	293	0.84	0.75	0.85	0.54	LCE	3.74	3.58	0.63	0.30
LCE	986	1389	2.90	2.93	0.62	0.49					
QUA	576	309	1.05	0.77	2.09	2.32					
ALB	614	151	1.03	0.71	4.35	3.52	LOP	3.92	2.39	2.21	1.29
LOP	1001	450	1.80	1.23	2.48	2.41					
other melanics	19	11	0.04	0.07	0.18	0.57					
combined melanics	3984	4637	10.92	6.80	0.67	0.18		10.92	6.80	0.67	0.18
total	43139	34648									

[a] See text for calculation of CPR.

Table 2. *Correlation coefficients between the principal phenotypes*

	POP	TYP	TRI	VIT	PRA	MAR	LAT	FLA	GIB	LCE	QUA	ALB
TYP	−0.37											
TRI	−0.12	−0.49[a]										
VIT	0.04	−0.31[a]	−0.01									
PRA	−0.11	−0.11	−0.01	0.05								
MAR	−0.12	−0.02	−0.06	−0.03	0.14							
LAT	−0.11	−0.19	0.03	−0.04	0.13	0.20[a]						
FLA	−0.15	0.12	−0.18	−0.25[a]	0.01	0.11	−0.03					
GIB	−0.13	−0.24	−0.02	−0.05	0.06	0.25[a]	0.16	0.14				
LCE	−0.31[a]	−0.35[a]	0.03	−0.12	−0.01	0.23[a]	0.18	0.16	0.56			
QUA	−0.20[a]	−0.07	−0.03	−0.13	0.02	0.17	0.19	0.15	0.18	0.23[a]		
ALB	−0.12	−0.27[a]	−0.03	0.14	0.31[a]	0.26[a]	0.33[a]	0.00	0.24[a]	0.21[a]	0.17	
LOP	−0.21[a]	−0.32[a]	0.02	−0.03	0.15	0.17	0.19	0.10	0.18	0.40[a]	0.28[a]	0.41[a]

[a] Coefficients significant at the 0.05 probability level.

comprise the TYP phenotype, whereas POP and the TRI-related phenotypes each contribute about 20%. Taken together, the melanics make up the remaining 10%.

(*c*) *Polymorphic balance and covariation between the phenotypes*

Correlation analyses (see table 2) show strong covariation between most phenotypes within the melanic category and negative associations between these and the non-melanic phenotypes POP, TYP and TRI. The three non-melanics are negatively associated with each other because they comprise such a large proportion of most populations (although only the relations between TYP on the one hand and TRI and VIT on the other reach formal significance).

The melanics do not behave as a single homogeneous group. Morphs within the group GIB, LCE, QUA, ALB and LOP are most closely associated, with the direction of all ten relations positive, six of them significantly so (see table 2). MAR shows positive associations with all of the other melanics, four of the seven being significant. LAT follows a similar trend to MAR, with which it is positively correlated, but fewer of the associations (only two of seven) are significant.

The first axis of a PCA of the dataset (see table 3)

Table 3. *Latent vectors of principal components extracted from morph freqency data*

component	1	2	3	4
eigenvalue	2.63	1.86	1.29	1.12
cumulative % of variation	20.27	34.60	44.53	53.05
POP	0.19	−0.38	−0.35	0.32
TYP	0.37	0.47	0.31	0.00
TRI	−0.13	−0.30	−0.27	−0.69
VIT	0.00	−0.43	0.15	0.26
PRA	−0.12	−0.14	0.50	−0.06
MAR	−0.16	0.12	0.11	0.27
LAT	−0.28	−0.05	0.25	−0.15
FLA	−0.04	0.42	−0.20	0.06
GIB	−0.35	0.10	−0.32	0.33
LCE	−0.47	0.19	−0.23	0.14
QUA	−0.24	0.22	−0.03	−0.30
ALB	−0.35	−0.18	0.39	0.18
LOP	−0.41	0.05	0.11	−0.04

shows highest factor loadings on TYP contrasted to a subset of the melanics that includes LAT, GIB, LCE, QUA, ALB and LOP. Clearly, the degree of melanism represents a primary axis in this polymorphism. Subsequent axes in this analysis are more difficult to interpret, but appear to differentiate primarily within the non-melanic category.

Taken together, the correlation and principal components analyses suggest that the eight melanic phenotypes fall naturally into two groups. GIB, LCE, QUA, ALB and LOP, which we will refer to as the 'core-melanics', are highly intercorrelated and occupy one extreme of the melanism axis. The other dark forms, which we will call the 'quasi-melanics', are less closely associated in their frequency patterns, either with each other or with the core-melanics. FLA in particular does not follow the trend of any other melanic and, with the exception of a negative correlation with VIT, is unrelated to any of the other phenotypes (see table 2). It also has the lowest factor loading on the melanism axis after VIT (see table 3).

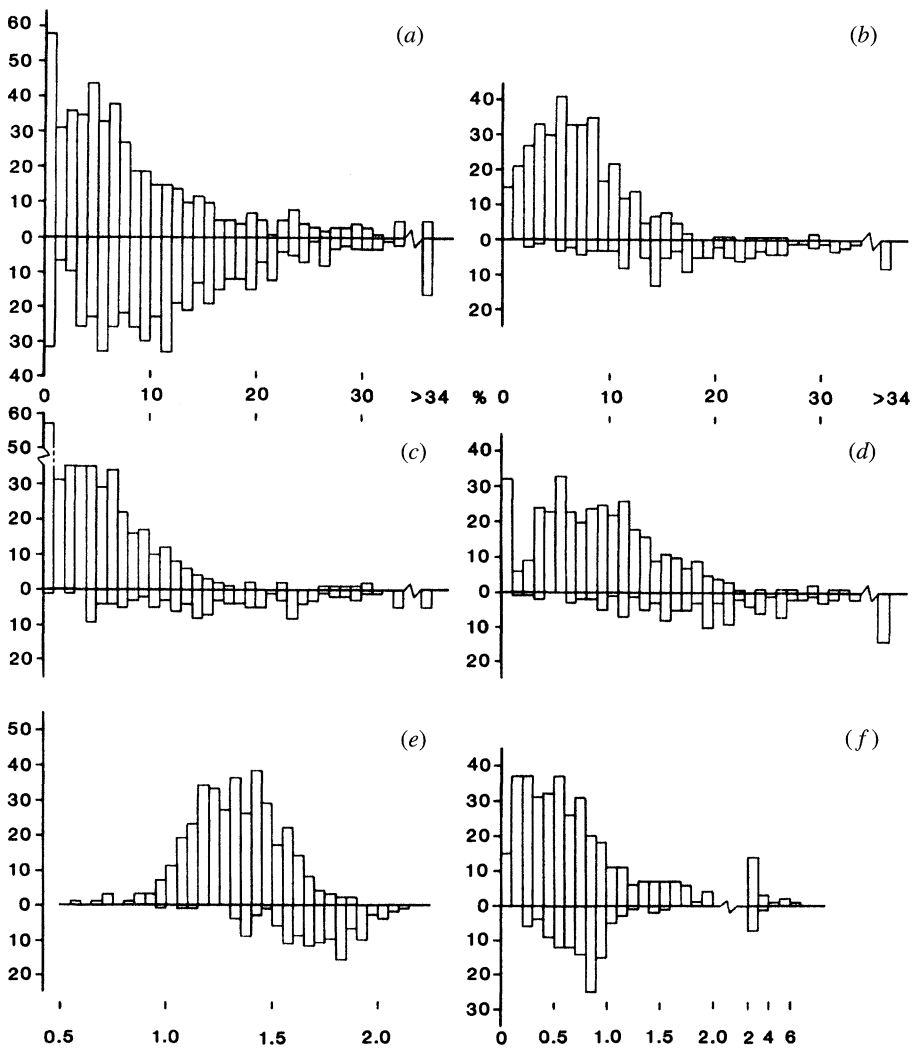

Figure 5. Contrasts in the distribution of melanic frequencies and related parameters between males and females and between rural and urban populations in England and Wales (number of populations on y-axis). (*a*) Contrast in melanic frequency (%) distribution between males (above x-axis) and females (below).(*b–f*) Contrasts between rural (above x-axis) and urban (below) populations: (*b*) melanic frequency, sexes combined; (*c*) melanic frequency, males only; (*d*) melanic frequency, females only; (*e*) polymorphic diversity, H; (*f*) CPR (see text for explanation).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

This almost certainly reflects its independent genetic control at the major colour locus, despite its visual similarity with other melanic phenotypes.

Some of the associations between phenotypes reflect common genetic control. For further analyses, it is convenient therefore to amalgamate some of the rarer phenotypes on the basis of their genetic affinity. Halkka *et al.* (1973) suggest that GIB and LCE are controlled by the same allele at the major colour locus and that the distinction between them is probably governed by linked modifier genes; accordingly, we consider them as a single phenotypic group. The same is true for QUA, ALB and LOP. The genetic basis of VIT and PRA is less certain, although the occurrence of intermediates between TRI and VIT suggest a common inheritance and there is good evidence from our own experiments that these two phenotypes are controlled in a similar way (Stewart & Lees 1988, also unpublished data). For many of the analyses here, TRI, VIT and PRA are therefore combined. The right-hand part of table 1 shows frequencies for these combined phenotypic groupings.

(*d*) *Interaction of the polymorphism with sex*

Examination of the raw data in table 1 reveals that most phenotypes are not represented equally in the two sexes. Overall, there is an excess of melanics in females compared with males (see figure 5*a*, table 6*a*). Other disparities between the sexes are apparent for other phenotypes.

Studies in other parts of the species range have reported widespread female limitation of certain morphs and reduced frequencies amongst males for other phenotypes. Elsewhere, we have suggested that a comparison of melanic frequencies in the two sexes can provide a strong indication of the underlying mode of genetic control in a particular population (Stewart & Lees 1987*b*).

Here, we extend the idea to consider all morphs. The relative penetrance of a phenotype in the two sexes can be expressed as a cross-product ratio of the frequencies in the males and females:

cross product ratio for morph x (CPR_x) =
(x ♂♂/total ♂♂) * (total ♀♀/x ♀♀).

The value of CPR ranges from 0 (not represented in males), through 1 (where frequencies in the two sexes are equal) to infinity. Values greater than 1 reflect higher frequencies in males compared to females; in practice, CPR values in large samples rarely exceed 3. The CPR for a particular phenotype is therefore a ratio of males to females, corrected for any imbalance in the sex-ratio of the sample as a whole.

It is immediately apparent from table 1 that the degree of penetrance in the two sexes, as measured by the CPR, varies considerably between phenotypes. TRI, when considered together with associated morphs VIT and PRA, approximates closely to a 1 : 1 sex ratio. TYP is also represented equally in the two sexes, although the CPR for the dark-TYP category demonstrates that these problematic specimens are found predominantly in the males.

Table 4. *Correlations between the frequency and* CPR *for each phenotype and between the frequency of the melanic phenotypes expressed as a percentage of the total melanic category and the frequency of all melanics combined*

(Based on combined data in Appendixes 1 and 2 ($n = 27$), correlation coefficients, r, and associated t values).

	between frequency and CPR for each phenotype		between frequency within melanic category and frequency of all melanics combined	
	r	t	r	t
POP	0.18	0.97		
TYP	−0.45	−2.74[b]		
TRI	0.08	0.43		
MAR	0.50	3.17[b]	−0.32	−1.86
LAT	0.32	1.84	−0.50	−3.18[b]
FLA	−0.20	−1.09	−0.19	−1.05
LCE	0.22	1.22	0.70	5.31[c]
LOP	0.10	0.53	−0.38	−2.23[a]
melanics combined:	0.51	3.28[b]		

[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$.

Particularly low CPR values for MAR and LAT reflect the greatly reduced penetrance of these phenotypes in males. A similar effect, although less severe, is evident in GIB and LCE. By contrast, the group comprising QUA, ALB and LOP are found more frequently amongst males.

A further complication concerns evidence that the expression of different phenotypes, as measured by the CPR values, varies between populations (see table 4). A positive correlation exists between frequency and CPR for the combined melanic category ($r = 0.51$, $P < 0.01$). Although the strict statistical significance of the relation is undermined by non-independence between frequency and CPR, the strength of the association does suggest that higher overall melanic frequencies promote enhanced expression of melanics in males. Of the constituent melanic morphs, only MAR shows a similar effect ($r = 0.50$, $P < 0.01$).

(*e*) *Comparison of rural and urban populations*

For ease of presentation, the data have been categorized into 20 rural regions and seven conurbations (Appendixes 1 and 2). Boundaries between rural regions were chosen using the Watsonian vice-county system (Dandy 1969), to define meaningful biogeographic units of comparable area.

Analyses in this section are based on the aggregated data for regions in Appendixes 1 and 2, rather than individual samples. As well as giving comparable means, this approach provides more realistic estimates of variance and the CPR for the rarer morphs, which are most susceptible to the stochastic effects of smaller sample sizes.

Summary statistics for the eight phenotypic groups and the combined melanic category are presented

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 5. *Summary frequency data for 20 rural regions and 7 conurbations in England and Wales*

(See text for calculation of CPR.)

	rural						conurbations					
	totals		frequency		CPR		totals		frequency		CPR	
phenotypic groups[a]	males	females	mean freq.	s.d.	mean	s.d.	males	females	mean freq.	s.d.	mean	s.d.
POP	7807	4087	21.39	3.55	1.58	0.35	2236	1257	16.66	3.78	1.37	0.32
TYP	15353	13591	52.37	6.29	0.91	0.06	5223	4282	40.92	4.54	1.00	0.09
TRI	5961	4738	18.99	5.56	1.02	0.16	2575	2056	23.34	5.95	0.97	0.07
MAR	17	287	0.53	0.22	0.04	0.04	22	143	0.85	0.56	0.11	0.14
LAT	91	593	1.21	0.46	0.12	0.10	66	329	1.91	0.87	0.15	0.06
FLA	145	453	1.10	1.13	0.79	1.30	149	229	1.40	1.03	2.06	3.72
LCE	396	566	1.68	0.85	0.58	0.33	888	1116	8.32	2.99	0.73	0.22
LOP	1062	486	2.72	0.94	2.25	1.51	1148	435	6.59	2.51	2.11	0.59
combined melanics	1711	2385	7.25	1.98	0.60	0.17	2273	2252	19.08	6.64	0.81	0.09
total	30832	24801					12307	9847				

[a] Phenotypic groups follow table 1.

Table 6. *Comparisons* (t-*test*) *of the distribution of melanic frequencies and related parameters between the sexes and between urban and rural populations in England and Wales*

comparison	mean % frequency		*t*	P
between males and females melanic frequency in:	males	females		
rural populations	5.70	9.14	9.53	< 0.001
urban populations	17.75	21.55	5.53	< 0.001
total data set	8.71	12.24	6.14	< 0.001
between urban and rural populations melanic frequency in:	urban	rural		
males	17.75	5.70	22.94	< 0.001
females	21.55	9.14	21.68	< 0.001
sexes combined	19.40	7.27	24.25	< 0.001
CPR	0.91	0.80	2.51	< 0.05
polymorphic diversity (*H*)	1.64	1.33	23.34	< 0.001
Chi squared values of test of association between melanic frequency and sex	1.74	2.09	2.07	< 0.05

separately in table 5 for the 20 rural and seven urban regions. In rural populations, the 20:50:20% balance between POP, TYP and TRI noted in table 1 is approximately maintained. The melanics together account for the remaining 5–10% of most rural populations. None of the individual phenotypes dominate the overall melanic category, most contributing around 1%, except the scarcer MAR and GIB which each comprise about 0.5% of most populations.

In urban populations, the melanic category rises to approximately 20%, but the relative contribution of the eight individual morphs is less even. Although all of the individual phenotypes show higher frequencies in comparison to rural populations, the combined melanic category becomes dominated by LCE. This difference is underlined by analysing how the relative contribution that each constituent phenotype makes to the overall melanic category varies as total melanic frequencies increase (see table 4*b*). Although the non-independence of the variables being compared means that the results should be viewed with some caution, it is clear that the relative frequency of the LCE group increases as total melanic frequencies increase, at the expense of the other groups, particularly LAT and LOP.

When the melanics are treated as a combined category, there are significant differences between rural and urban populations whether the sexes are considered separately or combined (see table 6*b* and figures 5*b–d*). Important differences are also apparent in terms of the degree of expression of the phenotypes in the two sexes, as reflected in their mean CPR values. With the exception of the LOP group, CPR values for individual melanic morphs are higher in urban populations (see table 5), a difference that is maintained when they are combined in one melanic category (see table 6*b* and figure 5*f*). A further interesting and highly significant difference is shown in polymorphic diversity, resulting from higher frequencies of most melanic morphs in urban populations (see table 6*b* and figure 5*e*).

Complete separation of rural and urban populations

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 7. *Discriminant function coefficients from canonical variate analysis, performed to separate: 367 rural from 122 urban populations; 367 rural populations in 20 regions; and 122 urban populations in 7 conurbations*

discriminant groups	urban versus rural	20 rural regions				7 conurbations			
component	1	1	2	3	4	1	2	3	4
eigenvalue	0.89	1.22	0.66	0.52	0.29	0.95	0.94	0.33	0.10
cumulative % of variance	100	44.95	63.83	77.26	86.46	39.38	78.47	92.09	96.18
POP	−10.97	−4.12	−34.81	4.22	−38.18	14.03	3.49	−32.23	22.56
TYP	−8.70	−2.61	−37.09	7.46	−23.56	3.24	−0.82	−16.10	21.57
TRI	−4.10								
VIT	−11.21	−15.38	−36.80	16.05	−27.89	12.20	6.90	−17.42	30.14
PRA	−2.43								
MAR	−16.86	8.07	−2.56	24.61	−28.38	−12.55	23.36	−37.48	73.14
LAT	−4.91	−11.01	−52.43	−36.22	−22.31	−47.32	29.82	−22.81	24.01
FLA	−4.04	35.35	−4.37	44.92	−38.46	12.04	−32.62	−23.69	65.31
GIB	18.18	−0.64	−40.61	−6.40	−29.88	8.90	−9.31	−24.66	16.85
LCE	7.05								
QUA	3.78								
ALB	14.89	−17.63	−35.14	4.45	−33.24	−0.54	8.48	−33.48	25.05
LOP	3.05								

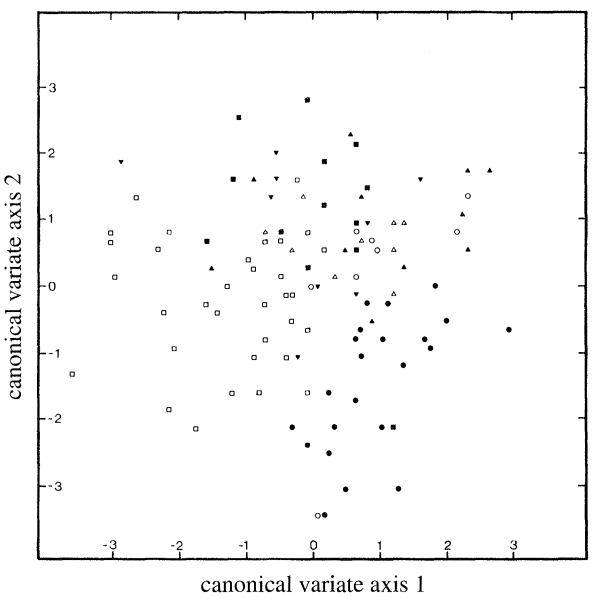

Figure 6. Biplot of first two axes in ordination of *P. spumarius* samples from 122 urban populations in England and Wales, using Canonical Variate Analysis on the percentage frequency of eight colour morph groupings (POP, TYP, TRI+VIT+PRA, MAR, LAT, FLA, GIB+LCE, QUA+ALB+LOP). Symbols refer to the seven conurbations: closed circle, London; open circle, Liverpool; closed square, Birmingham; open square, South Wales; closed upward-pointing triangle, Industrial northern England; open upward-pointing triangle, Manchester; closed downward-pointing triangle, northeast England.

was achieved along a single multivariate axis using Discriminant Function Analysis (see table 7*a*). Examination of the factor loadings shows a contrast between the core melanics and the rest of the phenotypes that recalls the primary melanism axis of the earlier PCA (see table 3). It is worth noting that MAR occupies a position on this axis at the opposite extreme to the other melanics, whereas LAT and FLA are intermediate; this provides further justification for treating these three phenotypes as quasi-melanics.

In an attempt to discriminate between the 20 rural regions, 85% of the variation in morph frequencies is explained by the first four axes (see table 7*b*). Although interpretation of the second and fourth axes is unclear, the discriminant function coefficients for the first show a high loading on FLA whereas the third represents LAT contrasted with FLA. Nearly 80% of the variation between the seven conurbations is explained by the first two axes, the first of which is dominated by the influence of LAT, whereas the second represents a contrast between FLA and MAR/LAT (see table 7*c*).

A biplot of the first and second urban axes (see figure 6) shows almost complete separation between sites in London and South Wales, primarily on the first axis. The London populations are characterized by high frequencies of FLA and low frequencies of LAT relative to South Wales. Separation of London and Birmingham sites along the second (FLA) axis is also almost complete. In fact, there is reasonable discrimination between sites in London and those in all the other conurbations using a diagonal between the two axes (i.e. on the basis of the combined influence of FLA and LAT).

(*f*) *Populations around single sources of air pollution*

Samples were collected in the vicinity of eight smokeless fuel works (three in South Wales, five on the South Yorkshire/Derbyshire/Nottinghamshire coalfield in northern England) in an attempt to establish whether other single sources of intense air pollution had a demonstrable effect on local melanic frequencies. A secondary aim was to compare the degree of expression of melanic genes in the males to that in previously reported populations around the phurnacite plant in the Cynon Valley, south Wales (Lees & Dent 1983).

Where samples were taken from more than one location around a coking plant, the data were tested for heterogeneity and combined where appropriate. The data for each site were compared with data from sites in the surrounding area; those in south Wales were compared with 30 other sites on the south Wales

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 8. *Frequency data for principal morph groups in samples around coking works*

For each site: upper row, percentage frequencies; lower row, CPR's. N_s Denotes number of samples combined, after heterogeneity tests. Local area comparison refers to comparison of melanic:non-melanic ratio at site with that in surrounding area. N_a Denotes number of sites in local area used for comparison. For sites in northern England, N_a refers to sites within 20 km radius. For sites in South Wales, N_a refers to 31 sites in table 2 of Lees *et al.* (1983) except Site 35 (Ynyshir), which was excluded because of its very large sample size (1776 individuals; approx 28% of total) and atypical morph frequency balance, which together had an undue effect on the combined data for South Wales.

													local area comparison			♂:♀ comparison	
coking plant	grid-ref		N_s	POP	TYP	TRI	MAR	LAT	FLA	LCE	LOP	TOTAL	Mels.	N_a	X^2	P	X^2	P
Nantgarw	3119	1856	3	21.0	44.2	14.5	0.7	1.5	2.6	8.9	6.5	538	20.3	30	0.29	N.S.	0.37	N.S.
				1.1	1.0	1.1	0.0	0.0	0.6	0.8	3.6		0.9					
Ty-Nant	3065	1855	2	20.9	44.6	17.0	1.4	1.9	3.6	7.5	3.1	359	17.5	30	2.77	N.S.	4.43	< 0.05
				1.1	1.4	0.6	0.0	0.0	0.3	0.7	9.9		0.6					
Coedely																		
works entrance	3017	1858	2	6.7	35.2	5.7	2.1	2.6	1.6	45.1	1.0	193	52.3	30	102.51	< 0.001	0.44	N.S.
				3.5	0.8	1.9	0.0	0.4	0.8	1.0	1.6		0.9					
field	3017	1859	1	9.2	39.1	16.1	0.0	2.3	3.4	24.1	5.7	87	35.6	30	10.44	< 0.01	0.22	N.S.
				6.5	0.8	0.9	N.A.	0.0	0.5	1.0	1.4		0.9					
west	3014	1862	1	22.4	35.3	19.8	0.0	2.6	1.7	11.2	6.9	116	22.4	30	0.09	N.S.	0.20	N.S.
				1.9	1.1	0.5	N.A.	0.5	0.0	0.6	3.0		0.9					
Ollerton	4650	3670	1	22.9	39.6	30.2	1.0	2.1	0.0	1.0	3.1	96	7.3	2	3.13	N.S.	2.36	N.S
				1.2	0.8	1.7	0.0	0.0	N.A.	0.0	N.A.		0.3					
Bolsover	4462	3715	3	27.1	35.2	18.8	0.6	2.6	0.4	6.4	8.9	531	18.8	4	8.76	< 0.01	0.85	N.S.
				1.3	1.1	0.7	0.0	0.1	0.7	0.3	9.7		0.8					
Grimethorpe	4410	4090	1	19.6	28.3	32.6	0.0	4.3	0.0	0.0	15.2	46	19.6	5	0.10	N.S.	0.94	N.S.
				5.6	0.4	1.4	N.A.	0.0	N.A.	N.A.	0.9		0.6					
Orgreave	4421	3879	1	14.7	28.4	21.6	1.1	1.6	1.1	19.5	12.1	190	35.3	4	35.04	< 0.001	2.38	N.S.
				0.9	1.1	1.8	0.5	0.2	0.5	0.7	0.9		0.7					
Askern	4556	4135	2	20.4	41.9	24.6	0.7	1.4	1.2	3.3	6.6	427	13.1	6	2.45	N.S.	9.60	< 0.01
				1.4	0.9	1.3	0.0	0.0	1.1	0.4	0.7		0.5					

coalfield (excluding the Cynon Valley) and those in northern England with all sites within a 20 km radius.

Table 8 shows that none of the populations exhibited melanic frequencies that even remotely approach the dramatically high frequencies found in the Cynon Valley, although three of the eight have frequencies significantly higher than the background mean for their area. Any local effect due to the plants at Nantgarw and Ty-Nant may have been eclipsed by the relatively high background melanic freqencies that characterize most populations in South Wales. In contrast, a significant local effect was found at Bolsover, even though melanic frequencies here are not appreciably higher than those around the two previously mentioned sites.

The most pronounced local effect is found at Coedely, where data from a limited number of samples suggest a direct influence on morph frequencies. Two combined samples taken either side of an access road and approximately 100 m east of the plant contained over 50% melanics. Allowing for the effect of the NW–SE orientation of the valley on the prevailing SW wind, this site was assumed to be directly downwind of the plant. Total melanic frequencies in two other samples, one also 100 m from the plant but at right angles to the wind direction and the other 100 m upwind from it, are 36 and 22% respectively. Melanic frequencies are significantly different from background levels in only the first two of these samples.

The expression of the melanic alleles is approximately equal in the two sexes for populations around six out of the eight coking plants (as shown by the CPR's and tested by chi-square in table 8). Significant under-representation of melanics in males occurred in two populations that were amongst the three showing the lowest overall melanic frequencies (< 18%).

(*g*) *Comparison with populations in southern Finland*

Morph frequencies in *P. spumarius* field populations have been reported from many parts of the species range, but nowhere more extensively than in southern Finland and Scandinavia. Table 9 contrasts data for southern Finland (combined from various sources; see legend) with analogous data for three broad groupings of British populations.

The data demonstrate the danger of comparing frequencies in different parts of a species range of phenotypes that are subject to variable degrees of penetrance. Overall melanic frequencies (i.e. with the sexes combined) in southern Finland are slightly higher than in rural British populations. However, comparisons are complicated by the distorting effect of differential expression of certain melanic genes in males, so that, for this purpose at least, data for females alone are better indicators of underlying allele frequencies. Female melanic frequencies in Finnish populations (18.2%) are nearly double those in rural British populations (9.6%) and closer to those in urban British populations (22.9%).

The composition of the melanic category is also very different in the two countries. More than 60% of female melanics in Finland are either MAR or LAT, contrasting with 37% in rural British populations. FLA is four times more frequent in British compared to Finnish females. Approximate percentage frequencies amongst females for the five melanic categories in table 9 are 25:35:5:15:20 in Finland and 10:25:20:25:20 in Britain, a difference which is highly significant when the totals in the five categories are compared ($\chi^2_{(4)} = 492.6$, $P < 0.001$). Interestingly, QUA + ALB + LOP is the only grouping whose contribution to the Finnish female melanic category is similar to that in both rural and urban British populations ($\chi^2_{(1)} = 1.62$ and 0.09 respectively, both $P > 0.05$).

Finnish populations are also characterized by widespread female limitation of certain morphs (primarily MAR and LAT, but also GIB and LCE) and reduced frequencies of most of the other melanic morphs in the males. This is reflected in the CPR's, which are zero or close to zero for all the melanic phenotypes, individually or combined. Only the QUA + ALB + LOP category occurs regularly amongst Scandinavian males, but even here at less than 0.5% compared to 3.4% in females.

In contrast, the same colour morphs occur regularly amongst males in rural British populations, although generally at lower frequencies than in females. Males comprise 5, 12, 26 and 56% of the MAR, LAT, FLA, and GIB + LCE phenotypes respectively and markedly exceed the females in the QUA + ALB + LOP category (CPR = 1.76). A comparison of the CPR's for the combined melanics for Finland (0.03) and rural Britain (0.58) indicates that male melanics are nearly 20 times as frequent in British populations.

(*h*) *Non-geographic variation*

(i) *Morph frequencies on different host-plants and in different habitats*

These analyses are necessarily somewhat crude because a substantial majority of samples was collected from one genus of host-plant (*Cirsium*) and the habitat classification is very simple. Nevertheless, in the light of host-plant and habitat effects found by previous workers (Whittaker 1968; Halkka & Mikkola 1977; Boucelham & Raatikainen 1984), it is important to establish whether such factors exert any substantial influence on morph frequencies that might complicate the interpretation of larger-scale geographical patterns.

Although there are no differences between the rural and urban populations in terms of the pattern of host-plants sampled, there are differences in the types of habitat sampled (see table 10, 'rural × urban' test); not surpisingly, more ruderal habitats, such as waste ground, roadside verges and disused industrial workings, were sampled in urban areas and there is significant under-representation of wet habitats. Accordingly, we analysed the rural and urban data separately (see table 10, 'within rural' and 'within urban' tests) and have restricted the analyses to comparing the different host-plant or habitat categories in terms of the distribution of sites across five equal melanic frequency groupings.

Using data on the list of host-plants sampled at each

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 9. *Comparison of morph frequency data for Britain with previously published data for Southern Finland*

	POP+TYP	TRI	MAR	LAT	FLA	LCE	LOP	melanics combined	total
Southern Finland[a]									
males	14975	456	3	1	0	0	74	78	15509
females	12790	482	801	1010	133	443	558	2945	16217
frequencies									
both sexes	87.51	2.96	2.53	3.19	0.42	1.40	1.99	9.53	
females only	78.87	2.97	4.94	6.23	0.82	2.73	3.44	18.16	
CPR	1.22	0.99	0.00	0.00	0.00	0.00	0.14	0.03	
Britain: 367 rural sites									
males	23160	5961	17	91	145	396	1062	1711	30832
females	17678	4738	287	593	453	566	486	2385	24801
frequencies									
both sexes	73.41	19.23	0.55	1.23	1.07	1.73	2.78	7.36	
females only	71.28	19.10	1.16	2.39	1.83	2.28	1.96	9.62	
CPR	1.05	1.01	0.05	0.12	0.26	0.56	1.76	0.58	
Britain: 122 urban sites									
males	7459	2575	22	66	149	888	1148	2273	12307
females	5539	2056	143	329	229	1116	435	2252	9847
frequencies									
both sexes	58.67	20.90	0.74	1.78	1.71	9.05	7.15	20.43	
females only	56.25	20.88	1.45	3.34	2.33	11.33	4.42	22.87	
CPR	1.08	1.00	0.12	0.16	0.52	0.64	2.11	0.81	
Britain: 36 sites in Cynon Valley, South Wales									
males	3370	900	30	24	264	3028	1867	5213	9483
females	2509	732	162	153	224	3537	324	4400	7641
frequencies									
both sexes	34.33	9.53	1.12	1.03	2.85	38.34	12.79	56.14	
females only	32.84	9.58	2.12	2.00	2.93	46.29	4.24	57.58	
CPR	1.08	0.99	0.15	0.13	0.95	0.69	4.64	0.95	

[a] Data combined from Halkka (1962, 1964; data for southwest Finland only), Halkka *et al.* (1970) and Boucelham & Raatikainen (1984).

Table 10. *Numbers of populations in five melanic frequency classes sampled from different host plants and different habitats*

combined melanic frequency classes (%):	rural					urban					chi-square comparison[a]		
comparison	0–5	5–10	10–15	15–20	> 20	0–5	5–10	10–15	15–20	> 20	rural × urban	within rural	within urban
principal host plant													
Cirsium sp.	94	132	50	11	5	3	13	25	22	39	$0.96_{(1)}$	$3.06_{(4)}$	$1.18_{(4)}$
other spp.	28	29	11	4	3	0	2	4	5	9	n.s.	n.s.	n.s.
host-plant diversity													
1 species	54	71	24	7	3	0	7	16	12	17	$0.01_{(2)}$	$2.80_{(8)}$	$7.91_{(8)}$
2 species	34	37	17	5	2	1	4	4	6	17	n.s.	n.s.	n.s.
3 or more species	34	53	20	3	3	2	4	9	9	14			
stability of habitat													
disturbed/ruderal	83	109	42	8	7	2	7	21	16	33	$5.23_{(1)}$	$3.67_{(4)}$	$8.25_{(4)}$
established	33	46	15	5	0	1	5	5	2	3	$p < 0.05$	n.s.	n.s.
wetness of habitat:													
wet habitats	31	38	14	2	0	0	1	4	1	3	$9.98_{(1)}$	$3.17_{(4)}$	$4.68_{(4)}$
dry habitats	85	117	43	11	7	2	12	22	17	33	$p < 0.01$	n.s.	n.s.

[a] 'Rural × urban' test refers to a comparison of the total number of rural and urban sites in the different habitat or host plant categories. 'Within rural' and 'within urban' tests refer to comparisons of the number of sites in the five frequency classes between the habitat or host-plant group(s). N.B. Expected values in some cells fall below 5; this sometimes produces over-inflated chi-square values making the test of equality more stringent. Degrees of freedom in parentheses.

site, we first compared samples where the primary host was *Cirsium* sp. with those collected principally from other species. Then we have compared data from samples collected off a single host species with samples taken from two or more species. Neither factor has any effect on the distribution of sites across the melanic frequency classes (see table 10). Similar analyses comparing samples taken from habitats of contrasting

levels of disturbance and wetness also failed to detect any significant effect on overall melanic frequencies.

(ii) *Temporal stability in morph frequencies*

Historical data on *P. spumarius* morph frequencies in Britain is sparce (Hutchinson 1963; Adenuga 1968; Whittaker 1968). Hutchinson (1963) contains data for two samples of *P. spumarius* from Cherry Hinton near Cambridge taken in 1920 and 1963. The site is a disused chalk pit that has been extensively recolonized by grasses, herbs and scrub. Precise relocation of the original site was facilitated by the fact that, given Hutchinson's description, the previous samples could have been taken only from a reasonably small and well defined area. We attempted to detect any change in morph frequency by re-sampling this population in 1983.

An obvious problem concerns the comparability of scoring techniques by different authors at different times. We have re-examined Hutchinson's original material taken from this site in 1963, which is currently housed in the Peabody Museum at Yale. Unfortunately, many specimens have incurred serious damage or have been lost, thus removing the possibility of re-scoring his samples. However, after inspection of those specimens which are both intact and labelled for phenotype, we are satisfied that his scoring was consistent with our own. Hutchinson included FLA and QUA under his *spumarius* (= TYP) category because he concluded that there was 'complete intergradation' between these forms. Although we would dispute this particular point, we have grouped our data in the same way in order to compare our data with his. The distinction makes little difference in this case because only 1 FLA and 1 QUA were recorded in our sample.

Hutchinson recorded 87 POP, 93 TYP, 44 TRI and 17 melanics in 1963. We recorded 33, 60, 13 and 7 of the same sequence of phenotypes in 1983, indicating that no significant change had occurred over the intervening 20 years ($X^2_{(3)} = 7.81$, $P > 0.05$). Hutchinson's sample from 1920 was really too small ($n = 49$) to justify statistical comparison with subsequent data, although he claimed that no significant change had taken place between 1920 and 1963.

8. DISCUSSION

Wherever the species has been studied, populations of *P. spumarius* are polymorphic for dorsal colour/pattern. Even on isolated islands (Halkka *et al.* 1970 1974*a*, 1975*c*; Brakefield 1990) or in extreme environments such as around the phurnacite plant in the Cynon Valley (Lees & Dent 1983), no populations have yet been reported that show fixation of any one allele. Large samples often contain all of the colour alleles, although many occur at very low frequencies. The universality and likely stability of the polymorphism strongly suggest the influence of some sort of balancing selection. However, the precise details of how the polymorphism is maintained remain elusive, even though a variety of both deterministic (Halkka *et al.* 1975*d*; Halkka & Mikkola 1977) and stochastic influences (Halkka *et al.* 1970; Brakefield 1990) have been suggested. To this extent, there are some parallels with other well-studied visible polymorphisms in the peppered moth *Biston betularia* (Lees 1981; Berry 1990), banded snails in the genus *Cepaea* (Jones *et al.* 1977), the two-spot ladybird *Adalia bipunctata* (Brakefield & Lees 1987) and candy-stripe spiders in the genus *Enoplognatha* (Oxford 1985; Oxford & Shaw 1986), where various influences have been suggested, the relative importance of which possibly varies between populations. Elsewhere (A. Stewart & D. Lees, unpublished data) we discuss the possible role of various evolutionary forces acting on the *P. spumarius* polymorphism; here we are concerned with establishing the features of the polymorphic balance in British populations.

Although the nature of the selection involved is uncertain, a primary objective of this survey was to put the populations in the Cynon Valley reported by Lees & Dent (1983) into a broader context. Is the extreme local adaptation, presumably to intense air pollution, found there repeated elsewhere or is it unique? These populations are remarkable not only for their very high melanic frequencies (approaching fixation of melanic alleles in some cases), but also for the composition of the melanic category, both between the phenotypes and between the sexes. Are these two features related or are all British populations characterized by increased penetrance of the normally female-limited morphs in males?

Despite a thorough search of several other sites that have been severely polluted by emissions from single sources of intense air pollution, we failed to find any *P. spumarius* populations with melanic frequencies that even remotely approach those recorded adjacent to the phurnacite plant in the Cynon Valley. Nevertheless, significant effects have been detected in populations around three of the eight coking works, whereas effects around at least two of the others may have been masked by the prevailing high melanic frequencies in the local area.

The degree of local response to pollution from these other pollution sources presumably results from the interaction of more than one factor; the nature and total load of pollutants emitted by the plant (generally unknown), gene flow from other populations, background morph frequencies in the surrounding area and local topography. Lees & Dent (1983) suggested that the steep sides of the Cynon Valley had the effect of concentrating emissions from the phurnacite plant there. The funnelling of the prevailing wind from the W and NW down the valley has produced clines in melanic frequencies away from the plant that were skewed downwind. There is a possibility that a similar topographic effect may be operating around the plant at Coedely. Lack of discernable local effects at the other two sites in South Wales (Nantgarw and Ty-Nant) may be partly because background melanic frequencies in populations on what was the South Wales coalfield are quite high (around 20%). A more level topography, allowing more efficient dispersal of emissions, may account for the lack of significant local

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

effects on morph frequencies around two of the plants in northern England and for the less pronounced response around the remaining two, as compared with the Cynon Valley.

The second most dramatic example of high melanic frequencies is found in a set of populations on wasteland in Cardiff docks and has been reported elsewhere (Lees & Stewart 1988). These sites are severely contaminated by dust and fine particulate material derived from large stockpiles of export coal. However, the unusually high melanic frequencies can not be attributed to this current effect without qualification, because the highest frequencies coincide with the position of a former smokeless fuel factory.

The survey reported in this paper demonstrates a significant 'urban effect' on morph frequencies. Populations in six of the seven conurbations exhibit melanic frequencies that are substantially greater than those in rural areas; rural frequencies are typically between 5 and 8%, rarely greater than 10%, whereas those for urban populations rise to between 15 and 20%. The exception is Liverpool, although no obvious reason for this is apparent, other than its coastal position and a possible ameliorating effect of the prevailing onshore winds.

Populations with high melanic frequencies, whether associated with single intense pollution sources or urban areas, also exhibit the features of the polymorphism found in the Cynon Valley; greatly increased penetrance of the normally female-limited phenotypes (MAR, LAT, GIB and LCE) in the males, resulting in near-equal melanic frequencies between the sexes, and predominance of LCE among melanics.

We originally posed the question of whether the first of these features was unique to the Cynon Valley populations, and perhaps other similarly polluted areas, or whether it constituted a characteristic British pattern of morph frequencies which could be detected even in rural populations. The occurrence in rural populations of male specimens of the morphs which elsewhere are strictly female-limited, such as MAR and LAT, suggests that a similar underlying genetic model for the polymorphism is operating across the whole of Britain. However, this must be qualified by the observation that the relative penetrance between the sexes of the different phenotypes varies between populations. There is good evidence that penetrance of the melanic alleles in males increases as overall melanic frequency increases, explaining why melanic frequencies in the Cynon Valley are approximately equal in the two sexes. This may also be so for individual melanic phenotypes, but low frequencies make this more difficult to demonstrate.

It is also apparent that the notion of some sort of general selection for melanism in *P. spumarius* is too simplistic. Frequencies of the different melanic phenotypes do not vary in parallel, as demonstrated by the subtle changes in balance within the combined melanic category as overall melanic frequencies change. MAR, LAT and FLA are relatively more common in rural populations where overall melanic frequencies are lower. The melanic category in urban and other populations subjected to elevated levels of atmospheric pollution tends to be dominated by GIB and LCE. This in itself is evidence that selection is not acting simply on the amount of melanin deposited in the cuticle; GIB and LCE are both predominantly black but with pale heads. Selection solely for darkness would result in highly polluted populations being dominated by the O-group of phenotypes (QUA, ALB & LOP). This is not the case even in the most severely polluted populations in the Cynon Valley.

The distinction between a core set of melanic phenotypes and the other melanics which are more loosely associated with them appears to be a useful one. MAR and LAT show no obvious association with urban areas and FLA in particular does not reflect trends in the core melanics. However, it is particularly interesting that variation in the frequency of these phenotypes provides the best discrimination between geographically separated groups of populations, whether these are the 20 rural regions or the seven conurbations.

With the exception of limited data from one site (Cherry Hinton), we have little information on the temporal stability of these patterns in morph frequency variation. Continued monitoring of populations on the island of Skokholm over more than ten years shows no sign of any significant morph frequency change and the closure of the phurnacite factory in the Cynon Valley in 1990 has yet to stimulate any major change in frequencies there (D. Lees, unpublished data). However, the response of polymorphisms in other insects to the improving air quality resulting from the Clean Air Acts of the 1950s and 1960s demonstrates that phenotype frequencies may respond very rapidly to environmental changes, through changes in the direction and intensity of selection. Declines in melanic frequencies in *B.betularia* (Cook *et al.* 1986; Clarke *et al.* 1990) and *A.bipunctata* (Brakefield & Lees 1987) have been as rapid as the presumed rate of increase in the mid-nineteenth century. Similarly, other polymorphisms unrelated to industrial effects have often shown a mixture of change and long-term stability in different populations (Oxford 1986; Cain *et al.* 1990; Cameron 1992).

The contrasts with the well-studied populations in Finland and other parts of northern and eastern Europe are striking. There are major differences in the overall frequency and composition of the melanic category; in relation to north-east European populations, British *P. spumarius* populations are characterized by relatively high frequencies of FLA and low frequencies of MAR and LAT. However, the most important feature of British populations is the increased penetrance of those phenotypes which are severely female-limited in other parts of the species range. The contrast between British and Finnish populations in this respect is particularly striking. Unfortunately, not all previous authors have reported their morph frequency data with the sexes separated. This means that any conclusions about the uniqueness of British populations must be treated with some caution. However, it is remarkable that no previous study has indicated a comparable breakdown in the female-limitation usually found in this subset of melanics.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Large areas of the species range have yet to be surveyed in any detail, ironically including those parts of continental Europe which are geographically nearest to Britain, where populations with a similar genetic background might most reasonably be expected to be found. These areas would repay future attention.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

APPENDIX 1

Numbers of 16 phenotypes in both sexes in 367 rural samples from 20 geographic regions of England and Wales

(For each region: males, upper line; females, lower line. ONM, other non-melanics; OM, other melanics.)

geographic region [Watsonian V.C.'s]	no of sites	POP	TYP	dark TYP	TRI	VIT	PRA	ONM	MAR	LAT	FLA	GIB	LCE	QUA	ALB	LOP	OM	total	mels	% mels
1 Northeast England	14	216	546	62	296	121	0	0	0	2	8	5	8	7	11	10	0	1292	51	4.8
[66–68]		139	444	10	82	168	3	0	8	19	4	3	8	6	2	5	0	901	55	
2 Lake District	18	340	617	71	308	166	1	0	0	6	6	5	7	17	13	31	0	1588	85	7.2
[69–70]		255	710	31	167	225	0	0	17	33	1	3	18	26	8	33	0	1527	139	
3 North Riding	17	299	525	54	208	71	1	0	0	9	3	0	6	10	18	20	0	1224	66	6.4
[62,65]		167	499	31	84	112	0	2	15	16	1	1	9	10	5	18	0	970	75	
4 West Riding	23	547	645	63	420	91	5	0	1	5	4	4	17	19	33	29	0	1883	112	6.3
[63,64]		322	594	33	224	132	15	0	14	26	5	5	19	7	2	19	0	1417	97	
5 East Riding & N. Lincs	20	521	1349	87	325	56	6	0	2	7	2	3	5	9	21	27	0	2420	76	5.1
[54,61]		211	1097	39	190	87	16	0	15	62	10	4	24	3	6	13	0	1777	137	
6 Lancs & Cheshire	23	523	1102	99	626	87	25	8	3	3	14	17	48	15	30	49	0	2649	179	8.4
[58–60]		316	833	47	419	119	29	9	26	58	12	21	49	5	13	24	0	1980	208	
7 North Wales	15	254	562	48	237	31	2	0	0	5	0	2	12	0	8	5	0	1166	32	3.6
[49–52]		156	475	8	119	50	4	0	6	19	1	3	3	2	3	4	0	853	41	
8 Mid Wales	24	461	1079	43	293	34	2	10	0	7	5	9	15	35	19	26	0	2038	116	6.8
[42,43,46–48]		306	826	9	173	41	11	1	8	45	10	8	16	13	7	17	0	1491	124	
9 Southwest Wales	13	229	555	24	153	12	0	4	1	4	2	2	16	12	12	6	0	1032	55	7.7
[44,45]		147	501	7	80	11	1	0	8	28	6	5	27	4	1	9	0	835	88	
10 South Wales	29	819	1322	49	355	73	1	11	3	13	9	14	67	45	26	53	2	2862	232	9.8
[35,41]		373	1080	29	230	118	19	2	37	48	45	8	71	21	2	20	1	2104	253	
11 Welsh Borders	16	238	368	24	119	25	3	0	0	0	3	4	10	10	11	19	0	834	57	7.3
[33,34,36,37,40]		122	398	5	68	48	9	0	4	17	9	2	13	5	4	2	0	706	56	
12 North Midlands	16	435	504	44	226	59	8	0	2	12	4	6	14	18	35	37	0	1404	128	10.3
[39,56,57]		229	427	12	156	97	16	0	17	51	4	11	23	7	4	10	0	1064	127	
13 South Midlands	12	349	522	43	144	45	1	0	0	4	6	9	11	5	8	16	0	1163	59	7.7
[32,38,55]		125	448	14	90	70	6	0	10	24	16	10	32	2	0	1	0	848	95	
14 The Fens	14	541	691	22	200	26	0	3	0	4	12	5	7	12	6	12	2	1543	60	5.1
[29,31,53]		217	693	24	108	76	6	0	3	22	28	4	10	4	1	9	0	1205	81	
15 N. & W. Home counties	15	404	626	20	162	35	5	0	2	6	7	6	12	16	25	33	0	1359	107	10.1
[20,22–4,30]		185	694	19	81	119	8	0	25	38	28	7	39	13	2	5	0	1263	157	
16 East Anglia	20	406	725	13	191	6	6	1	1	0	19	0	2	10	5	19	0	1404	56	7.8
[19,25–28]		226	731	12	145	36	12	0	18	17	84	3	8	13	5	7	0	1317	155	
17 Devon & Cornwall	25	203	497	49	139	38	3	2	1	1	7	3	3	15	6	4	3	974	43	4.7
[1–4]		124	566	11	63	67	1	0	8	10	5	6	10	1	2	1	1	876	44	
18 Somerset, Dorset	16	457	755	22	177	17	4	9	0	1	11	5	6	12	9	15	5	1505	64	6.8
[5–9]		204	667	15	109	65	10	4	17	16	42	6	17	10	3	8	0	1193	119	
19 Hants, Sussex	21	352	902	44	186	23	1	25	1	2	13	3	11	15	9	13	3	1603	70	8.9
[11–14]		152	946	39	136	65	10	10	19	29	95	6	28	8	2	20	5	1570	212	
20 Kent, Surrey	15	213	481	26	83	22	1	0	0	0	10	5	12	8	11	17	0	889	63	10.3
[15,18,21]		111	511	28	54	67	11	0	12	15	47	5	21	13	1	6	2	904	122	
all rural sites	367	7807	14373	907	4848	1038	75	73	17	91	145	107	289	290	316	441	15	30832	1711	
combined		4087	13140	423	2778	1773	187	28	287	593	453	121	445	173	73	231	9	24801	2385	

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

APPENDIX 2

Numbers of 16 phenotypes in both sexes in 105 urban samples from 7 conurbations in England and Wales

(For each region: males, upper line; females, lower line. ONM, other non-melanics; OM, other melanics.)

conurbation	no of sites	POP	TYP	dark TYP	TRI	VIT	PRA	ONM	MAR	LAT	FLA	GIB	LCE	QUA	ALB	LOP	OM	total	mels	% mels
1 London	22	470	833	43	335	17	6	6	5	2	42	24	115	32	26	69	1	2026	316	18.6
		232	837	52	234	84	26	0	15	9	90	43	189	39	3	19	2	1874	409	
2 Liverpool	7	155	414	41	264	44	10	1	0	3	2	13	20	7	7	9	0	990	61	6.8
		77	304	15	135	65	13	2	2	11	1	10	16	2	2	8	0	663	52	
3 Manchester	9	196	456	40	280	29	12	7	1	5	11	30	40	18	24	31	0	1180	160	15.3
		139	291	24	183	53	9	6	12	31	5	16	49	6	10	22	0	856	151	
4 Midlands (Birmingham, Leicester, Nottingham, Coventry)	13	187	287	18	168	32	39	0	0	9	3	6	28	2	21	45	0	845	114	15.5
		88	306	9	131	57	23	0	15	35	10	12	41	1	6	13	0	747	133	
5 Industrial north (Leeds, Sheffield, Bradford, Doncaster, Huddersfield)	11	221	289	5	155	36	10	0	0	3	5	12	29	10	33	30	1	839	123	16.3
		114	198	5	74	60	9	0	8	26	2	10	41	2	4	13	0	566	106	
6 Industrial north-east (Tyneside, Teesside)	9	168	393	53	223	62	0	0	8	7	17	24	63	23	40	60	0	1141	242	21.6
		94	274	15	116	120	1	0	15	35	1	8	66	9	20	23	0	797	177	
7 South Wales	34	505	1710	53	557	16	2	3	7	33	53	56	305	172	109	248	1	3830	984	27.1
		322	1482	19	403	58	3	2	55	134	92	32	424	71	22	98	0	3217	928	
all urban sites combined	105	1902	4382	253	1982	236	79	17	21	62	133	165	600	264	260	492	3	10851	2000	20.2
		1066	3692	139	1276	497	84	10	122	281	201	131	826	130	67	196	2	8720	1956	