Biological Journal of the Linnean Society (1993), *50:* 235–253. With 3 figures

Trans-continental visible morph-frequency variation at homologous loci in two species of spider, *Enoplognatha ovata s.s.* & *E. latimana*

G. S. OXFORD[1]* AND P. R. REILLO[2]

[1]*Department of Biology, University of York, York YO1 5DD, U.K.*
[2]*Rare Species Conservatory, 1222 'E' Road, Loxahatchee, Florida 33470, U.S.A.*

Received 19 October 1992, accepted for publication 22 December 1992

The spiders *Enoplognatha ovata s.s.* and *E. latimana* are sibling species which share a number of visible genetic polymorphisms. Data on colour and black-spotting morph frequencies in these species have been collected from 67 sites in western Europe. Sixty nine percent of the collections contained both species. In all adequately-sized samples, both species were polymorphic for colour and, in general, exhibited the same rank order of morphs *lineata* and *redimita*. (The top dominant morph, *ovata*, has not been found in *E. latimana*). Colour-morph frequencies are not correlated between species in sympatric populations from mainland Europe and from a previously studied area in Pembrokeshire, South Wales. Although associations with certain climatic variables are evident in *E. ovata* they are not consistent between transects, making their biological significance unclear. For black spotting, *E. ovata s.s.* is nearly fixed for spotting throughout mainland Europe but is highly variable in the Pembrokeshire populations. *E. latimana* is polymorphic in both areas. In Europe, spotting frequencies in *E. latimana* show significant associations with climatic factors but, again, their biological significance is not obvious. In *E. ovata s.s.* the variance in both colour and spotting frequencies among populations in Pembrokeshire is significantly greater than that in the whole of mainland Europe. The implications of these and previous results are considered in the context of the persistence of visible polymorphisms across species and the forces which determine morph frequencies in local populations.

ADDITIONAL KEY WORDS:—spiders – visible polymorphism – geographical variation – homologous polymorphisms – selection – genetic drift.

CONTENTS

INTRODUCTION

From a genetical point of view, the most thoroughly studied spider is undoubtedly the cosmopolitan Theridiid, *Enoplognatha ovata* (Clerck). This

*Correspondence to G. S. Oxford.

0024–4066/93/011235+19 $08.00/0

© 1993 The Linnean Society of London

species exhibits a striking colour and pattern polymorphism which has attracted attention since at least the 1930s (Bristowe, 1931). The polymorphism comprises three major morphs, *lineata* with a plain yellow/cream abdomen, *redimita* which has, in addition, a pair of dorsolateral carmine stripes and *ovata*, in which the entire dorsal shield is carmine (Oxford, 1983: Plate 1). These morphs are genetically determined by alleles at an autosomal locus (Oxford, 1983; Reillo & Wise, 1988a). Much of the recent work on this species has investigated the spatial and temporal distributions of colour morphs, and the factors controlling them, on a number of geographical scales in Britain (Oxford, 1976, 1985a, 1991; Oxford & Shaw, 1986), North America (Reillo, 1989; Reillo & Wise, 1988b, c; Wise & Reillo, 1985) and northern continental Europe (Hippa & Oksala, 1979, 1981).

Among the vast majority of populations examined, *E. ovata* colour-morph frequencies vary significantly over distances of tens of metres, and these differences persist over many generations. Perturbation experiments on two continents have indicated that powerful natural selection is apparently not involved in determining local morph frequencies, and have given rise to the suggestion that genetic drift, both continuous and intermittent, might be of major importance (Oxford & Shaw, 1986; Reillo & Wise, 1988b). Investigations of two other genetic characters, the regulation during development of red pigment deposition in morphs *redimita* and *ovata*, and black spotting (Oxford, 1983), in some of the same populations, support this interpretation (Oxford, 1985b, 1989). On a larger spatial scale (tens to hundreds of kilometres), patterns of morph-frequency variation suggest that natural selection acts on the colour locus. For example in Britain, consistent, but very weak, clines in colour-morph frequencies across the country indicate an association with certain climatic variables, notably wetness indices (Oxford, 1985a). Similar morph-frequency clines are evident in eastern North America (Reillo, 1989) and possibly in northern continental Europe (Hippa & Oksala, 1979).

It is possible that selective forces affecting the colour polymorphism on the large scale are also acting at the local level but are too weak to be detected easily. The identification by Hippa and Oksala (1982, 1983) of a number of sibling species within *E. ovata* affords an unusual opportunity to detect the operation of even weak selection acting locally (Endler, 1986; Oxford, 1991). At least two of the species within the *E. ovata* group, *E. ovata s.s.* and *E. latimana* Hippa and Oksala, share the same colour, regulatory and spotting polymorphisms (Oxford, 1992). The geographical distributions of these two species overlap considerably (Oxford & Reillo, in press). If it can be shown that visible morph frequencies co-vary between species within a number of mixed-species populations then, barring hybridization, selection alone must be responsible (Endler, 1986). An initial study of sympatric populations in Pembrokeshire, South Wales (Oxford, 1991) did not suggest that colour or spotting frequencies were correlated between species, but the number of suitable populations examined was small.

In the present paper we investigate colour and spotting morph frequencies in *E. ovata s.s.* and *E. latimana* along two large-scale transects running approximately north-south through western continental Europe. We use the data on patterns of morph-frequency variation, both within and between species, to address a number of questions relating to the maintenance of the visible

polymorphisms and the factors which control morph frequencies: (1) what is the incidence of polymorphisms in the two species, and are there consistent morph-frequency trends within populations? (2) are there patterns in colour-morph and spotting frequencies within species which correlate with geographical and/or environmental variables? (3) are there qualitative trends in morph and cocoon frequency patterns within and between species? and (4) irrespective of any geographic trends, are there quantitative associations between morph frequencies in the two species in sympatric populations? Finally, we examine the overall levels of morph-frequency variability within species over different geographical scales.

METHODS

Spiders were collected from a total of 67 sample sites between 3 and 15 August 1991. Sampling sites are shown in Figure 1 and describe two major transects. Transect 1 from the Netherlands south to northern Italy, a distance of about 900 km, consisted of sites 1–32 (referred to as data set 1). The sampling regime here was to collect two or three samples from sites perhaps one or two kilometres apart and then move on about 50–60 kilometres and collect another two or three. The second transect, about 750 km in length, ran from the French/Spanish border northwards to the Cherbourg peninsula and comprised sites 43–67 (set 3). In addition to these, sites numbered 33 to 42 (set 2) were sampled along the south coast of France during transit between transects. For sets 2 and 3, where spiders were more difficult to locate, the sampling regime used for transect 1 had to be abandoned. For comparative purposes, data collected in Pembrokeshire, South Wales in 1990 (Oxford, 1991) are also used (set 4).

At the time of sampling, females of both species were mature and had established themselves in rolled or folded foliage of broad-leaved plants guarding, or preparing to produce, a cocoon (Oxford, 1993). At each site an area of suitable vegetation was located and thoroughly searched for rolled leaves. The vegetation sampled was often on open road verges but in some cases occurred on scrubland, along hedgerows, or in open woodland. The area searched was not constant but depended on spider densities. Any males seen wandering in the general vegetation were also taken but were not used in the analyses.

At each sample site, all the spiders seen were caught and preserved in 70% alcohol. Time constraints dictated that at site 3 alone, females were segregated in the field according to whether or not they possessed cocoons. The red pigment of morphs *redimita* and *ovata* is known to fade in alcohol so spiders from each site were separated on the basis of colour morph within twelve hours of capture. Subsequently, in the laboratory, individuals were further scored for species, sex and black-spotting phenotype. An estimate was also made of the proportion of spiders that had produced cocoons on the basis of abdominal size. Individuals were classified as +, ? and −, where '+' indicated a spider with a small abdomen (had produced a cocoon), '−' indicated a spider with a very swollen abdomen (had not yet produced a cocoon), and '?' denoted a spider with an intermediate-sized abdomen. Data from site 3 were used to test the efficacy of this classification and indicate the status of spiders denoted as '?'.

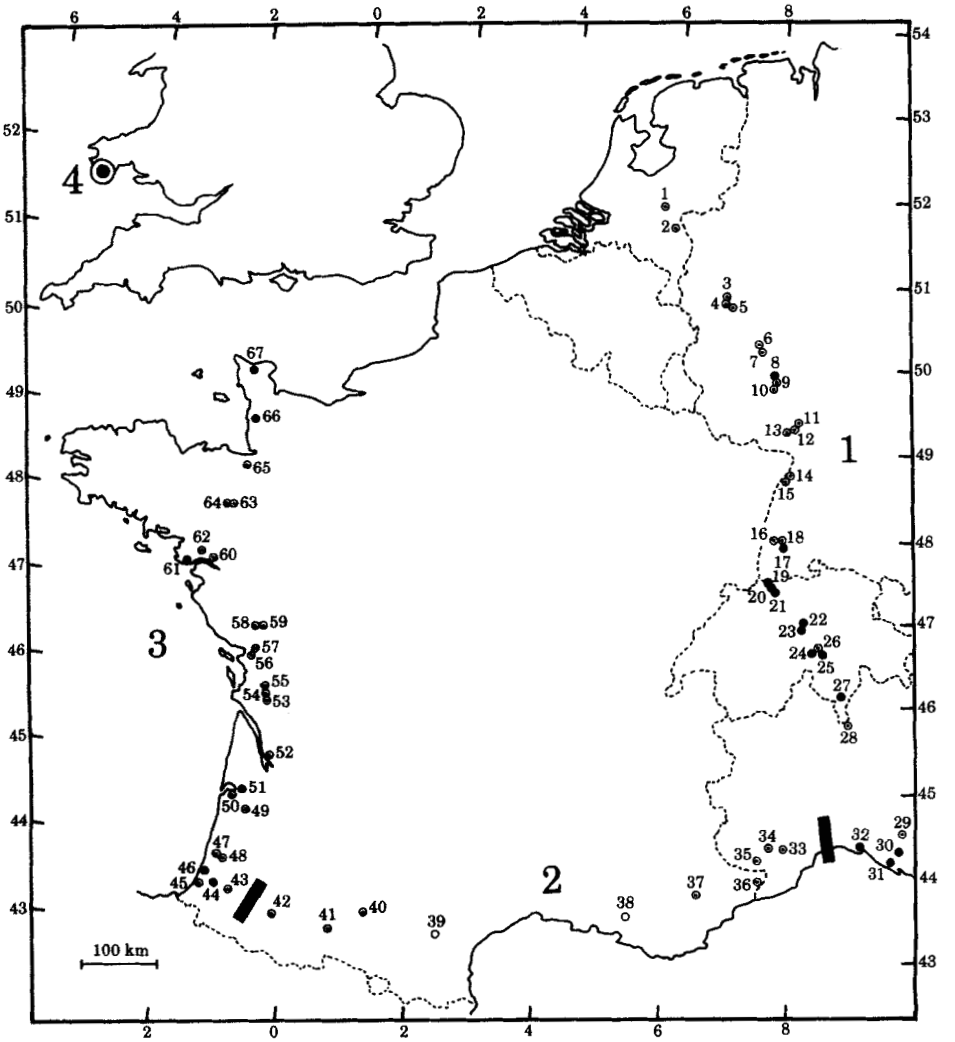


Figure 1. Distribution of sampling sites in western Europe. Solid circles represent samples with only *E. ovata s.s.*; open circles samples with only *E. latimana*, and open circles with a central dot samples containing both species. The four data sets mentioned in the text are 1: The Netherlands to north Italy, 2: south of France, 3: western France and 4: Pembrokeshire.

Information on the latitude, longitude and altitude (m) of sample sites was obtained from large scale national maps. The following climatic variables were estimated from smaller scale maps published by the World Meteorological Organization (World survey of climatology, 1970, 1977): mean January temperature (°C), mean July temperature (°C), annual rainfall (decimetres) and, for all countries except Italy, mean annual insolation (hours . 10^{-2}). Associations between morph frequencies and these potential explanatory variable(s) were sought by logistic regression methods using the GLIM statistical package. It was assumed that the distribution of morph frequencies was binomial. Models containing various combinations of explanatory variables were assessed with likelihood ratio techniques using the relative values of deviance produced by the GLIM system. Other statistical analyses were performed on

Minitab. Comparisons of variances in morph frequencies among geographical areas were made using a jackknife method (Layard, 1973), since F-tests, and techniques for comparing more than two variances (e.g. Bartlett's test), are very sensitive to deviations from an assumed underlying normal distribution (Box, 1953; Miller, 1974).

RESULTS

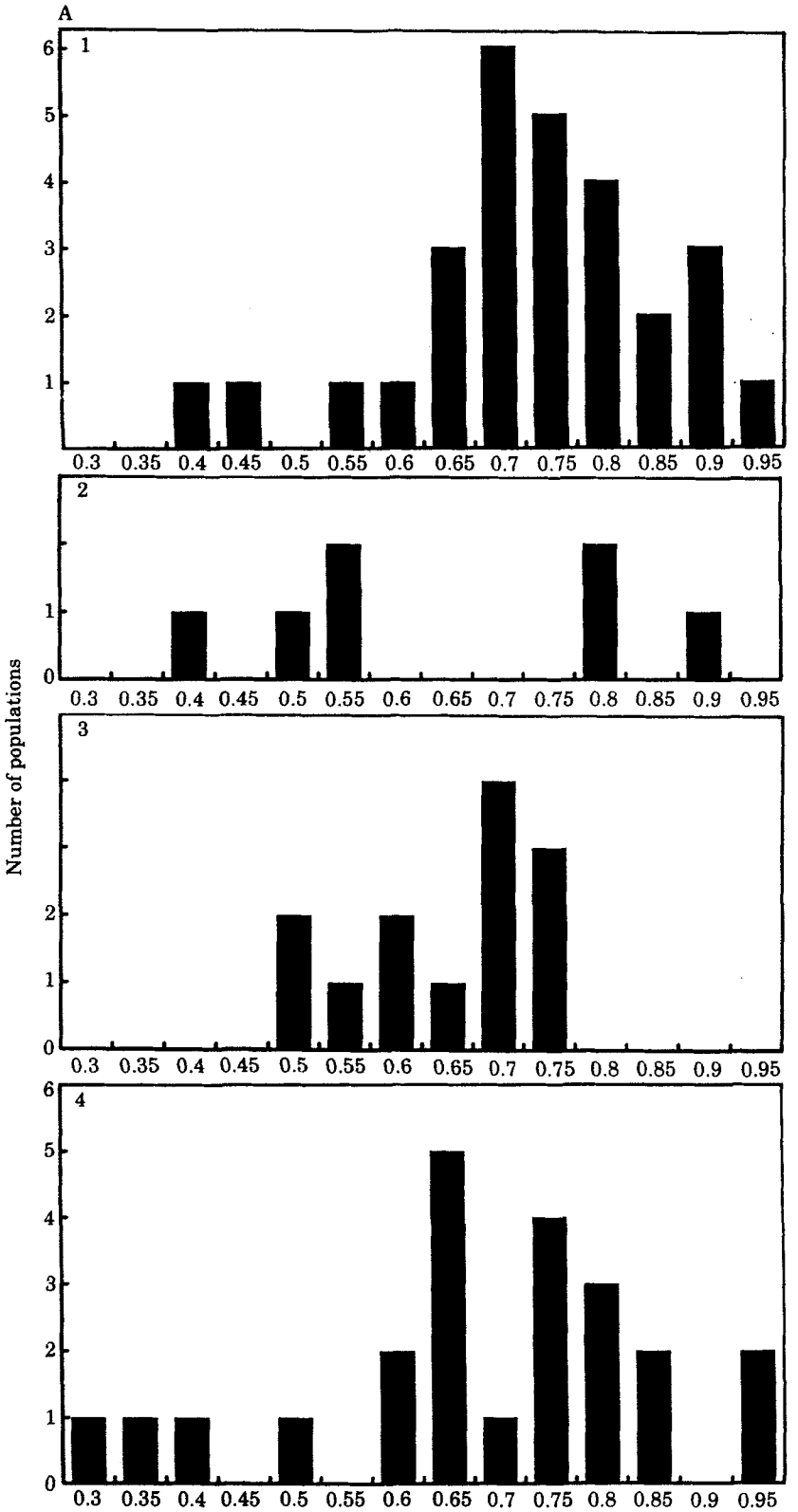
In total, 5537 individuals were collected from the 67 sample sites, comprising 4768 *E. ovata s.s.* and 769 *E. latimana* (Appendix). Two sites contained only *E. latimana*, 19 only *E. ovata s.s.* and the remaining 46 (69%) were mixed, to varying extents (Appendix, Figure 1). The two samples consisting of *E. latimana* alone were of one and two spiders respectively, and on this basis can hardly be designated true single species populations. Of the samples with *E. ovata s.s.* alone, five consisted of six or fewer animals but the majority were represented by 50 or more individuals (Appendix). Clearly, both species are very widely distributed in western Europe (see also Oxford & Reillo, in press) and often occur in sympatric populations. In the area considered here, single species *E. ovata s.s.* populations are not uncommon whereas single species *E. latimana* populations are rare or absent, a situation reflecting that found in Britain (Oxford, 1991, 1992 and unpubl.).

The efficacy of assessing cocoon status from abdomen size was tested by one of us (GSO) scoring blind individuals of known status from site 3. Of 100 individuals scored in this way, 89 were correctly classified. Eight out of the 11 designated as '?' were without cocoons, the other three had cocoons but were large and may have been about to produce a small second cocoon, as happens in a low percentage of cases. In the analyses that follow, therefore, the '?' category has been amalgamated with '−'.

Incidence of polymorphism

In the 65 samples containing *E. ovata s.s.*, six were monomorphic for colour, five for *lineata* and one for *redimita*. The median sample size for these six was three. In populations represented by sample sizes of 10 or more, all of which were polymorphic (Fig. 2A), the rank order of morphs was *lineata* > *redimita* > *ovata* in 43 out of 48 (89%). *Lineata* had the highest, or equal highest, frequency in 47 out of 48 samples (98%). *E. latimana* was polymorphic for colour in 30 out of a total of 48 collections (62%), but only for the *lineata* and *redimita* morphs; the *ovata* morph has yet to be found in this species. Of the 19 samples consisting of 10 or more individuals, all were polymorphic and 15 (79%) had *lineata* > *redimita*. Two of the collections in which *redimita* predominated (sites 42 and 46) were relatively large (sample size was 58 in each). Thus, in both species, the colour polymorphism is present in all but the smallest samples and, in polymorphic populations, *lineata* tends to predominate.

The black spots on the abdomen of the two species vary in both presence/absence and, if present, in number. In *E. ovata s.s.* presence/absence appears to be determined by a major locus with number of spots controlled by polygenes (Oxford, 1989). Here we consider only the presence and absence of spots since spotted individuals almost invariably possessed the full spot complement in both


A
1
2
3
4
Number of populations
0.3 0.35 0.4 0.45 0.5 0.55 0.6 0.65 0.7 0.75 0.8 0.85 0.9 0.95

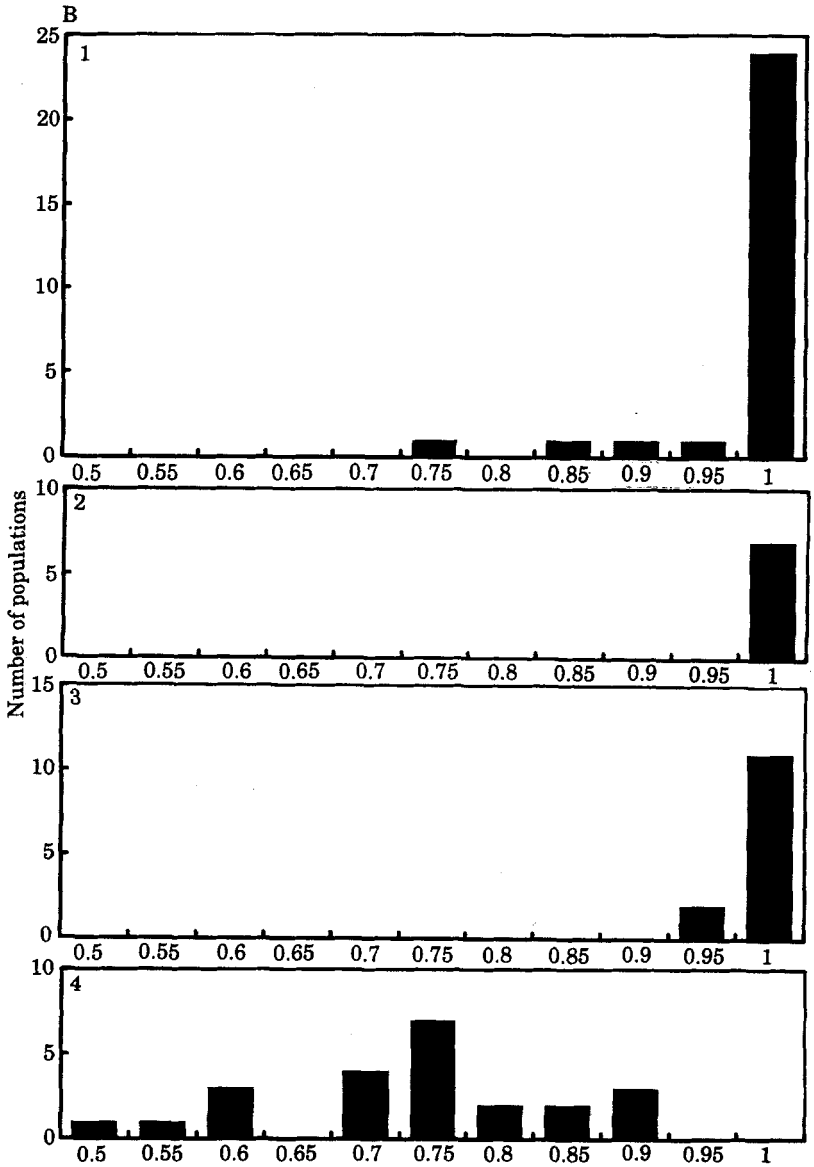


Figure 2. Distribution of (A) *lineata* and (B) spotting morph frequencies in *E. ovata s.s.* samples of ten or more individuals from the four data sets. The values of morph frequencies shown are mid-points for each column.

species. In *E. ovata s.s.*, presence of spotting shows a very skewed distribution with 81% of all samples fixed for this character (Fig. 2B). All samples of less than ten individuals were fixed. Of the polymorphic populations, only three had proportions of spotted individuals less than 95% (77%, 87% and 90%, respectively) and these were all geographically clustered in central Switzerland (sites 24–26, Fig. 1). *E. latimana* was much more variable in this respect. Of the 48 samples, 21 were fixed for spotted or unspotted phenotypes, although many of these collections comprised very few individuals. If samples of less than ten

individuals are excluded, the distribution is strikingly bi-modal. Four of these samples have spotting frequencies greater than 90% and are all geographically located near the coast in north-western Italy and south-eastern France (sites 29, 33, 35, and 37, Fig. 1). The major mode in the distribution is at spotting frequencies between 15 and 40%.

Patterns in morph frequencies associated with environmental variables

Data sets 1 and 3 were analysed separately for possible associations between the frequency of the *lineata* colour morph and six or seven environmental variables. In set 1, insolation information was not available for Italy and this variable has been omitted. For *E. ovata s.s.*, the only explanatory variable which significantly reduced the scaled deviance for this transect was mean January temperature, which had a negative effect on *lineata* frequency (Table 1a). Three large collections from Switzerland were made at altitudes of 1100 m and would be expected to have a considerable influence on this relationship. However, omitting these sites reduces but does not eliminate the highly significant association with January temperature. In addition, longitude now enters the equation but is barely significant (Table 1b). The sign of the relationship between frequency *lineata* and longitude is also negative. For *E. latimana* there are fewer samples and many are very small and therefore assigned low weights in these analyses. None of the possible explanatory variables reduced significantly the scaled deviance, although the single variable which reduced it most was January temperature.

Analyses of data set 3, from western France, showed that a combination of mean July temperature and altitude achieved the greatest reduction in scaled deviance for *E. ovata s.s.* (Table 1c). The frequency of the *lineata* morph is negatively related to both. The significance of altitude is lost, however, if the highest site in the transect (67 at 150 m) is omitted (Table 1d). For *E. latimana* none of the explanatory variables reduced the scaled deviance significantly.

There is too little variation in the frequency of black spotting in *E. ovata s.s.* to ask whether specific environmental variables may influence this character in Europe. For *E. latimana* samples in set 1, altitude produces the greatest single drop in scaled deviance. This is further reduced by the addition of July temperature to the equation. However, the significance of the latter is marginal (Table 1e). For both, the relationship with frequency of spotting is positive. In data set 3, January temperature has the major single effect on scaled deviance. Altitude also enters the equation but with very marginal significance (Table 1f). The sign of the relationship is positive with January temperature and negative with altitude.

Qualitative associations between morph and cocoon frequencies within and between species

Tests for independence of characters, colour (*lineata*/others) vs. spotting (presence/absence), colour vs. cocoons (presence/absence) and spotting vs. cocoons, within species were assessed in 2×2 tables using Fisher's exact test, since the numbers involved were often small. Trends among the contingency tables were tested using Cochran's Y (Everitt, 1977), which takes account of differences in sample size. Similar analyses were made on colour and cocoons

TABLE 1. Logistic regression analyses of morph frequencies in *E. ovata s.s.* and *E. latimana*

E. ovata s.s.—*lineata* morph			Variable fitted	Scaled deviance	d.f.
(a) Data set 1—all samples					
			none	141.4	31
			January	76.6	30
Regression parameters:					
constant	1.09 ± 0.04	$P \ll 0.001$			
January	-0.15 ± 0.02	$P \ll 0.001$			
(b) Data set 1—samples at 1100 m altitude omitted					
			none	91.3	28
			January	70.4	27
			January + longitude	66.5	26
Regression parameters:					
constant	2.18 ± 0.56	$P < 0.001$			
January	-0.12 ± 0.03	$P < 0.001$			
longitude	-0.14 ± 0.07	$P = 0.05$			
(c) Data set 3—all samples					
			none	70.0	24
			July	34.7	23
			July + altitude	29.1	22
Regression parameters:					
constant	7.82 ± 1.08	$P \ll 0.001$			
July	-0.38 ± 0.06	$P \ll 0.001$			
altitude	-0.003 ± 0.001	$P = 0.002$			
(d) Data set 3— sample 67 omitted					
			none	67.7	23
			July	30.0	22
Regression parameters:					
constant	8.23 ± 1.20	$P \ll 0.001$			
July	-0.40 ± 0.06	$P \ll 0.001$			
E. latimana—spotted morph			Variable fitted	Scaled deviance	d.f.
(e) Data set 1					
			none	82.0	18
			altitude	28.6	17
			altitude + July	25.4	16
Regression parameters:					
constant	-8.47 ± 3.23	$P < 0.01$			
altitude	0.005 ± 0.001	$P \ll 0.001$			
July	0.35 ± 0.17	$P < 0.05$			
(f) Data set 3					
			none	30.6	18
			January	23.7	17
			January + altitude	19.8	16
Regression parameters:					
constant	-4.35 ± 1.36	$P < 0.01$			
January	0.71 ± 0.25	$P < 0.01$			
altitude	-0.01 ± 0.005	$P < 0.05$			

between species in sympatric populations. Tests involving spotting within *E. ovata s.s.* were only possible for a very small number of sites, since this character was often fixed, and none was possible between species.

Within *E. ovata s.s.* none of the comparisons between colour and cocoon status was significant (number of tests, $n = 47$) and there was no sign of a trend

(Cochran's Y = 0.04, n.s.). Likewise, colour vs. spotting comparisons ($n = 6$) were not significant. In one site, 26, there was a highly significant association between presence of spots and presence of a cocoon ($P = 0.0025$) and overall there was a significant trend in the same direction ($n = 6$, Cochran's Y = 2.45, $0.02 > P > 0.01$). However, removal of site 26 reduces Cochran's Y to 0.64 (n.s.). In *E. latimana*, two out of 23 comparisons of colour vs. spotting were significant (with $P = 0.033$ and 0.013, respectively) and there was no trace of a trend (Cochran's Y = −0.023, n.s.). For spotting vs. cocoons, only one out of 21 tests was significant (with $P = 0.021$) and, again, no trend (Cochran's Y = 0.054).

Associations between species in mixed-species samples were tested for colour and cocoons. For colour, only two of 46 comparisons were significant (with $P = 0.048$ and 0.0025, respectively) and there was no trend (Cochran's Y = 0.037). For cocoons, 16 out of 46 comparisons had *E. ovata s.s.* with a significantly higher proportion of cocoons than *E. latimana*, and 19 of the rest were in the same direction. Overall, the trend was highly significant (Cochran's Y = 12.15, $P \ll 0.001$). This difference in phenology between the species is now well established (Oxford, 1992) and is also reflected in the proportions of males, which die soon after mating. Eighteen *E. ovata s.s.* males and 17 *E. latimana* males were found, making up 0.4% and 2.2% of the species totals, respectively ($\chi^2_{(1)} = 34.5$, $P \ll 0.001$).

Morph-frequency variation within sympatric populations

Although nearly 70% of samples contained *E. ovata s.s.* and *E. latimana*, one or both were often in very low numbers. Only 15 samples comprised 10 or more individuals of both species. Angularly transformed frequencies of the *lineata* morph in the two species in these populations are not significantly correlated (r = 0.36, d.f. = 13). In a previous study, Oxford (1991) sampled five mixed species populations from Pembrokeshire, South Wales, in which at least 10 individuals of each species was represented. If these are analysed together with the European samples the correlation remains non-significant (r = 0.33, d.f. = 18) (Fig. 3A). For black spotting, as mentioned above, there is very little variation in *E. ovata s.s.* in Europe. The correlation between angularly transformed spotting frequencies in the two species is not significant (r = 0.42, d.f. = 13). However, the addition of the five Pembrokeshire samples produces a significant correlation (r = 0.54, d.f. = 18, $0.02 > P > 0.01$). Inspection of the scatter plot (Fig. 3B) suggests that this correlation results from combining two sets of heterogeneous data, since all the Welsh samples show low spotting frequencies in both species.

Variance in morph frequencies over different geographical scales

The sampling programme used for the first transect (data set 1) was designed so that the variance in morph frequency within a locality could be compared with that among different localities, and thus allow an assessment of the degree of spatial differentiation. A similar sampling strategy was adopted in a recent survey of both species in Pembrokeshire (Oxford, 1991), although there the sampling localities were not on a linear transect. For all samples containing 10 or

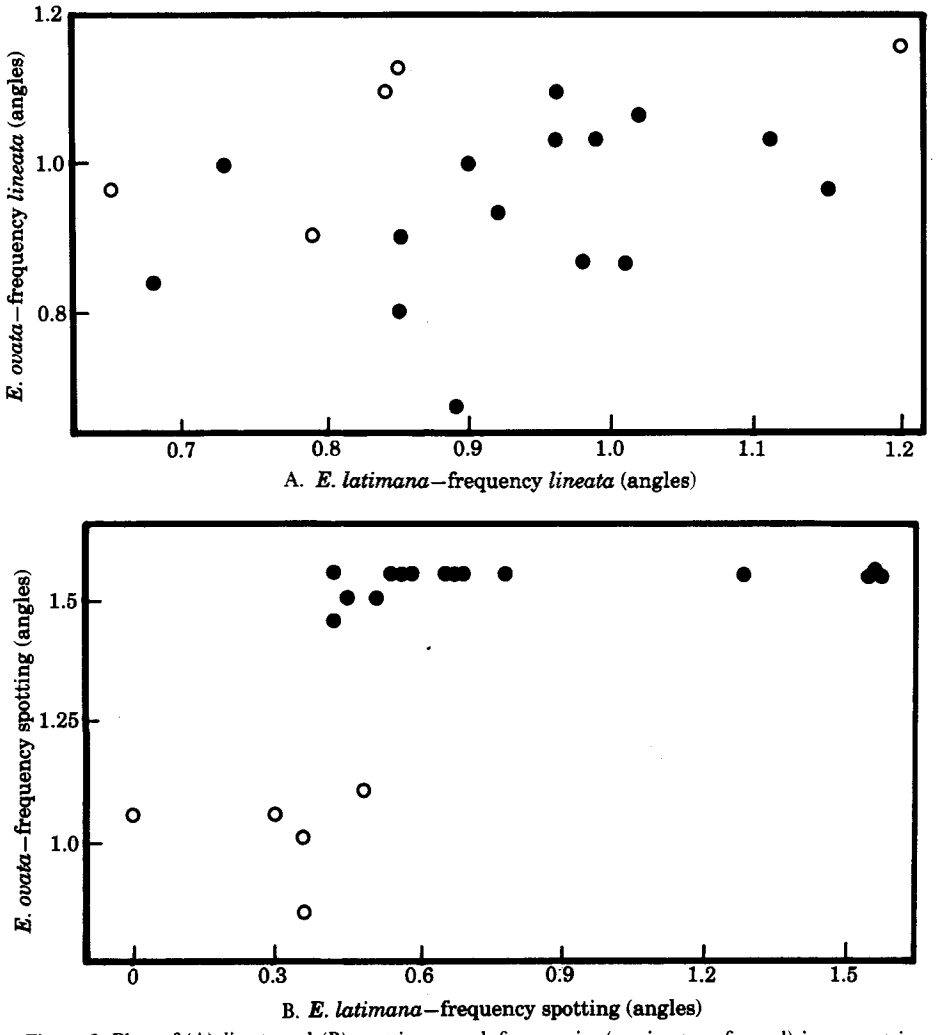


Figure 3. Plots of (A) *lineata* and (B) spotting morph frequencies (arcsine transformed) in sympatric populations of *E. ovata s.s.* and *E. latimana*. The open circles represent data from Pembrokeshire.

more individuals, arcsine transformed morph frequencies were subjected to one-way analyses of variance.

For the frequency of the *lineata* morph in *E. ovata s.s.*, there was significant geographical differentiation in Pembrokeshire ($P = 0.014$) but not in transect 1 on the continent ($P = 0.079$). In the latter, the last triad of samples (sites 29, 30 and 32) was taken from a much larger area ('locality') than the others and may not be comparable. If this is omitted the significance falls even further ($P = 0.384$). For spotting, geographical differentiation in transect 1 was highly significant ($P \ll 0.001$), a result due entirely to the uniformly low frequency in the high altitude Swiss sites 24–26. If these are omitted the test is no longer significant ($P = 0.151$) and is even less so if the more scattered triad is also ignored ($P = 0.301$).

It is also instructive to compare the overall variance in visible morph frequencies among populations of each species between Pembrokeshire and the

TABLE 2. Comparisons of population variances in Pembrokeshire vs. mainland Europe using the jackknife method

	Minimum sample size	Number of samples Pembroke	Europe	*P*	Rank[1]
Frequency of the *lineata* morph:					
E. ovata					
	$n \geq 5$	26	53	n.s.	P > E
	≥ 10	23	48	n.s.	P > E
	≥ 20	21	40	0.04	P > E
	≥ 30	16	36	0.004	P > E
	≥ 50	9	27	0.004	P > E
E. latimana					
	$n \geq 5$	12	29	n.s.	E > P
	≥ 10	8	19	n.s.	P > E
	≥ 20	6	11	n.s.	P > E
	≥ 30	5	9	n.s.	P > E
Frequency of the spotted morph:					
E. latimana					
	$n \geq 5$	12	29	0.003	E > P
	≥ 10	8	19	< 0.001	E > P
	≥ 20	6	11	0.001	E > P
	≥ 30	5	9	0.005	E > P

[1]P > E indicates that the variance is greater in Pembrokeshire than in Europe, and *vice versa*.

whole of Europe (data sets 1–3). The estimated variance within these two areas will be an amalgam of true differences in morph frequencies among individual populations and a component resulting from sampling errors. The latter is expected to become smaller as sample size increases. Variances between the European and Welsh samples were therefore compared using a jackknife method (Layard, 1973) for all populations represented by sample sizes of ≥ 5, ≥ 10, ≥ 20, ≥ 30 and, for *E. ovata s.s.*, ≥ 50. The results are shown in Table 2. It is clear that for colour in *E. ovata s.s.*, the variance among populations is significantly higher in Pembrokeshire than in Europe and that the significance increases as smaller sample sizes are excluded, suggesting that sampling errors are not the cause. As mentioned earlier, spotting in this species in Europe is very skewed. Inspection of Fig. 2B shows that samples from Pembrokeshire (set 4) are much more variable for spotting than those from continental Europe; a statistical test is unnecessary. In *E. latimana* the variance in *lineata* frequency does not differ significantly between the two areas, but in larger samples there is a tendency for Pembrokeshire to have the higher value. Spotting in *E. latimana* is significantly more variable on the continent than in South Wales, although as noted above, the spotting distribution in the former is bimodal.

DISCUSSION

All research with the *Enoplognatha ovata* system has shown that the colour polymorphism is nearly ubiquitous when sample sizes are adequate. There are few cases of monomorphic populations (Oxford, 1985a; Reillo, 1989) and these are all fixed for the bottom recessive *lineata* allele. They tend to occur on the

edges of the species range where phases of extinction and recolonization might be expected to be more prevalent. The rank order of colour morphs *lineata* > *redimita* > *ovata* predominating in the present samples is also found in the vast majority of populations elsewhere (e.g. Hippa & Oksala, 1979; Oxford, 1985a; Reillo, 1989). If monomorphic colonies result from the founder effect it is almost inevitable, therefore, that they are fixed for the *lineata* morph. The pattern of colour-morph frequency variation in *E. latimana* is less well known, but again, all adequately sampled populations are polymorphic (see also Oxford, 1991, 1992). The rank order of morphs in this species is the same in the majority of populations and, as in *E. ovata s.s.*, *lineata* predominates. The only major difference between the species is that the *ovata* morph is apparently absent in *E. latimana*.

The polymorphism, and the consistent rank order of morphs, are therefore common to both *E. ovata s.s.* and *E. latimana*. The mean frequencies of the *lineata* morph in the two species are also similar in Europe and Pembrokeshire combined, although the tendency for *E. latimana* to have a lower frequency is almost significant (all samples ≥ 10, $t = 1.94$, d.f. 49, $P = 0.059$). The species differ in a number of ways including isozyme variation, reproductive strategies and phenologies (Hippa & Oksala, 1982; Snazell, 1983; Oxford, 1992 and unpublished), as well as in the sexual characters used to separate them (Hippa & Oksala, 1982). This suggests that they have been evolving separately for a considerable time and yet both have presumably retained the colour polymorphism and associated morph-frequency characteristics from a common ancestor. Indeed, both species also share the spotting polymorphism, as described here, and the regulatory polymorphism which controls the timing of red-pigment deposition during development in morphs *ovata* and *redimita* (Oxford, 1992).

Environmental factors do not easily explain the geographical variation in colour- and spotting-morph frequencies. For the frequency of *lineata* in *E. ovata s.s.*, the major factor in data set 1 is January temperature and is such that frequency decreases in areas with warmer winters. In data set 3, however, July temperature is the main variable to emerge, with *lineata* frequency decreasing in areas with warmer summers. None of the analyses of colour frequencies in *E. latimana* was significant. For spotting, *E. ovata s.s.* cannot be analysed since the character is fixed or nearly fixed in the majority of populations. This character in *E. latimana* showed significant positive associations with altitude in data set 1 and January temperature in data set 3. Thus, for a particular character, different explanatory variables were identified on the two transects, which is not surprising given the contrasts in topography and proximity to the Atlantic Ocean, which will have an ameliorating influence on climate. However, it is not at all obvious what, if anything, is the biological significance of these associations.

Within species, colour and spotting phenotypes, and colour and the presence of a cocoon, vary independently, as has been found elsewhere (Oxford, 1991). For cocoons and spotting in *E. ovata s.s.*, very few comparisons were possible but there is a significant trend in the direction of spotted individuals having a higher probability of possessing a cocoon at the time of sampling. However, the significance of this trend depends critically on a highly significant association in one population. A similar, highly significant trend was also found in this species

in the Pembrokeshire study (Oxford, 1991). No such association is apparent in *E. latimana*. The reason for the relationship between spotting and cocoons is not clear, but might possibly involve faster growth rates at some stage in the life cycle as a result of thermal melanism. The only significant qualitative trend between species was that *E. ovata s.s.* was reproductively more advanced than *E. latimana* at the time of sampling since a higher proportion of females had cocoons, and a lower proportion of males was caught. This corroborates data from other studies (Hippa & Oksala, 1982; Snazell, 1983; Oxford, 1991, 1992). There are no obvious geographical trends in this respect, which suggests that the difference between species is present, at least qualitatively, throughout their area of sympatry in western Europe.

Of particular interest from the point of view of common selection acting on visible polymorphisms are the comparisons of morph frequencies between species in sympatric populations. The present study has yielded 15 sites at which the sample size of both species exceeds 10 individuals. For these sites, plus the five from Pembrokeshire, Wales, analysed earlier (Oxford, 1991), the correlation for colour is positive but not nearly significant. Spotting does show a significant positive correlation between species ($0.02 > P > 0.01$); however, the significance is a result of pooling data from Pembrokeshire, where both species tend to be less spotted, and Europe where both are relatively more spotted. The two sources of data are clearly heterogeneous and the positive relationship should be treated with caution. Worthy of note, however, is that the few European sites with lower frequencies of spotting in *E. ovata s.s.* also exhibit lower frequencies in *E. latimana*, suggesting that a relationship may exist; only further sampling over smaller geographical scales will resolve the matter. At present there is no compelling evidence that a common selective force is determining colour morph or spotting frequencies in mixed species populations. However, the species do differ phenologically and in other ways, and the possibility of species-specific selective agents cannot be categorically eliminated.

Finally we address the variance in morph frequencies within and among geographical areas. For *E. ovata s.s.* at least, the variance in colour and spotting morph frequencies among samples from Pembrokeshire is greater than that among those taken throughout western continental Europe. This is remarkable. Data on morph frequencies in Europe were collected from populations along a U-shaped route over a linear distance of more than 2000 km and enclosing a land area of some 735 000 km^2. The transects covered a large altitude range and encompassed a number of different climatic regimes. The Pembrokeshire data were gathered from populations within a land area of less than 140 km^2, which is extremely uniform in both topography and climate. Samples here were taken on a much finer scale than in continental Europe, but it is not obvious how this *per se* could produce the observed results. The situation in Pembrokeshire is matched on a finer scale still by populations of *E. ovata s.s.* in Nidderdale, U.K. (Oxford & Shaw, 1986), suggesting that the phenomenon of marked local differentiation is not unique.

What light do these observations, and those from previous studies, shed on the processes which (1) determine morph frequencies in local populations, and (2) lead to the persistence of the polymorphism? Here we will concentrate on the colour polymorphism, which is the better known. In the present survey we have failed to identify the action of significant, common selective forces operating on

the species in sympatric populations. This result is consistent with conclusions drawn from other surveys and from experimental work. Oxford & Shaw (1986) analysed data from a long-term study of colour-morph frequencies in *E. ovata s.s.* in Nidderdale and came to the conclusion that stochastic phenomena—continuous and/or intermittent genetic drift—probably play a major role in population differentiation. Similar views were expressed by Reillo & Wise (1988b) after conducting a large-scale perturbation experiment on the same species in North America. The much greater phenotypic variance in colour morph frequencies found in Pembrokeshire compared with continental Europe is entirely compatible with this conclusion. It seems unlikely that the Pembrokeshire situation is a result of strong, local selection, since it is very difficult to imagine the kind of force, biotic or abiotic, which might operate on this scale but not across a continent. Given the results of previous work, it is possible that the differences have an historical basis. If, in the past, the Pembrokeshire populations have been more disturbed compared with the average European population, then stochastic events could have led to a greater degree of local genetic differentiation. If the situation in Nidderdale is typical, populations may take a very long time (of the order of 100 years) to regain some sort of regional equilibrium after serious perturbations (Oxford, 1989).

Although we have argued for a non-selective interpretation of genetic differentiation at the local level, this cannot be the whole story; we have also to consider the persistence of the polymorphism. Three phenomena need to be accommodated: (1) the retention of the polymorphism in two sibling species, (2) the very consistent rank order of morphs in both *E. ovata s.s.* and *E. latimana*, and (3) the weak, but statistically significant, correlations of morph frequencies with climatic variables across the European continent, and within Britain (Oxford, 1985a). There are at least three hypotheses which might be invoked to explain the maintenance of the colour polymorphism in *Enoplognatha*: (1) the alleles at the colour locus are wholly neutral, (2) selection acts on pleiotropic effects of the colour alleles, but the colours themselves are of little or no selective consequence (i.e. are neutral or nearly so), or (3) selection acts on the colour polymorphism *per se* but only under certain conditions.

Consider the phenomena requiring explanation in the light of these hypotheses. If alleles are neutral in all respects than the rank order of morph frequencies would have to be a function of forward and backward mutation rates. For the rank order of morphs observed in most local populations to be determined by mutation rates, large effective population sizes are required and these must persist over very long periods of time (Crow & Kimura, 1970). Furthermore, they are very sensitive to perturbation. This does not seem to match the situation in *Enoplognatha*, although even a small amount of migration among sites will considerably increase the effective population size (Crow & Kimura, 1970: 268–270). Given the population structure of the spider, on the mutational balance hypothesis, one would expect many more populations to be far from equilibrium, with high frequencies of the redimita and orata morphs. The persistence of the polymorphism in two sibling species is also difficult to explain if alleles are truly homologous and neutral (Golding, 1992). Consistent, large scale patterns of morph-frequency variation could result from passive diffusion of neutral alleles but, at least in Britain, a selective interpretation fits the observations more satisfactorily (Oxford, 1985a).

The second hypothesis has been advanced by Goodhart (1987) as a possible explanation for the maintenance of visible polymorphisms in *Cepaea*. If alleles at the colour locus in *Enoplognatha* are selectively maintained, but for reasons other than their effects on the appearance of the spider, and if this selection is reasonably strong then perturbation experiments (Oxford & Shaw, 1986; Reillo & Wise, 1988b) should have revealed evidence for it. In any case, the problem of local selection operating much more effectively in Pembrokeshire than in Europe, remains. If selection acting on pleiotropic effects of the colour alleles is very weak, then this hypothesis would be difficult to distinguish from the final one.

Here it is envisaged that some sort of polymorphism-promoting selection, either acting on the colour variation *per se* or on pleiotropic effects of the colour alleles, comes into play only under the specific circumstances in which morph frequencies stray towards the extremes. These extremes, according to the rank order of morphs, will be when *lineata* approaches c. 0.5 or 1. At intermediate morph frequencies selection might be extremely weak and stochastic processes (continuous and intermittent genetic drift) become paramount in determining frequencies in local populations. This hypothesis alone tackles the intriguing paradox of selection apparently operating on large geographical scales and at the species level, but not in local populations. It explains why in most populations most of the time the demonstration of selection is so difficult.

It follows from this argument that if population sizes and gene flow are sufficiently small to allow population differentiation as a result of drift, then the selection operating at extreme morph frequencies must be very powerful in order to preserve the polymorphism in such a high proportion of populations. If the nature of the selection is essentially intra-specific, e.g. a rare male effect, one would not predict correlations between morph frequencies in sympatric populations. However, if selection is inter-specific, e.g. apostatic selection imposed by predators, then some sort of interaction in terms of morph frequencies might be expected (Clarke, 1969). The data presented here suggest little evidence for the latter. The basic hypothesis of selection at extreme frequencies can be tested by observations on replicated, near monomorphic experimental populations; these are urgently needed. Whether the spotting polymorphism can be explained in a similar way remains to be seen.

ACKNOWLEDGEMENTS

We thank Peter Woodford (National Meteorological Library) and Ann Inie (Librarian, Department of Geography, University of Leeds) for supplying, or allowing access to, climatic and topographical information. We are also very grateful to John Byrne and Mark Williamson for patiently assisting with the statistical analyses and to Laurence Cook, Robert Cameron and an anonymous referee for helpful comments on the manuscript. James Merryweather helped with the construction of Figure 2. The work reported here was supported by the National Geographic Society (Grant No. 4405–90).

REFERENCES

Box GEP. 1953. Non-normality and tests on variances. *Biometrika*, **40:** 318–335.

Bristowe WS. 1931. Notes on the biology of spiders—V. *Theridion ovatum*, Clerck, its habits and varieties. *Annals and Magazine of Natural History* **8:** 466–469.

Clarke B. 1969. The evidence for apostatic selection. *Heredity* **24:** 347–352.
Crow JF, Kimura M. 1970. *An Introduction to Population Genetics Theory.* New York: Harper & Row.
Endler JA. 1986. *Natural Selection in the Wild.* Princeton: Princeton University Press.
Everitt BS. 1977. *The Analysis of Contingency Tables.* London: Chapman & Hall.
Golding B. 1992. The prospects for polymorphisms shared between species. *Heredity* **68:** 263–276.
Goodhart CB. 1987. Why are some snails visibly polymorphic, and others not? *Biological Journal of the Linnean Society* **31:** 35–58.
Hippa H, Oksala I. 1979. Colour polymorphism of *Enoplognatha ovata* (Clerck) (Araneae, Theridiidae) in western Europe. *Hereditas* **90:** 203–212.
Hippa H, Oksala I. 1981. Polymorphism and reproductive strategies of *Enoplognatha ovata* (Clerck) (Araneae, Theridiidae) in northern Europe. *Annales zoologici Fennici* **18:** 179–190.
Hippa H, Oksala I. 1982. Definition and revision of the *Enoplognatha ovata* (Clerck) group (Araneae: Theridiidae). *Entomologica scandinavica* **13:** 213–222.
Hippa H, Oksala I. 1983. Cladogenesis of the *Enoplognatha ovata* group (Araneae, Theridiidae), with description of a new Mediterranean species. *Annales Entomologici Fennici* **49:** 71–74.
Layard MJW. 1973. Robust large-sample tests for homogeneity of variances. *Journal of the American Statistical Association* **68:** 195–198.
Miller RG. 1974. The jackknife—a review. *Biometrika* **61:** 1–15.
Oxford GS. 1976. The colour polymorphism in *Enoplognatha ovatum* (Clerck) (Araneae: Theridiidae)—temporal stability and spatial variability. *Heredity* **36:** 369–381.
Oxford GS. 1983. Genetics of colour and its regulation during development in the spider *Enoplognatha ovata* (Clerck) (Araneae: Theridiidae). *Heredity* **51:** 621–634.
Oxford GS. 1985a. A countrywide survey of colour morph frequencies in the spider *Enoplognatha ovata* (Clerck) (Araneae: Theridiidae); evidence for natural selection. *Biological Journal of the Linnean Society* **24:** 103–142.
Oxford GS. 1985b. Geographical distribution of phenotypes regulating pigmentation in the spider *Enoplognatha ovata* (Clerck) (Araneae: Theridiidae). *Heredity* **55:** 37–45.
Oxford GS. 1989. Genetics and distribution of black spotting in *Enoplognatha ovata* (Clerck) (Araneae: Theridiidae), and the role of intermittent drift in population differentiation. *Biological Journal of the Linnean Society* **36:** 111–128.
Oxford GS. 1991. Visible morph-frequency variation in allopatric and sympatric populations of two species of *Enoplognatha* (Araneae: Theridiidae). *Heredity* **67:** 317–324.
Oxford GS. 1992. *Enoplognatha ovata* and *E. latimana*: a comparison of their phenologies and genetics in Norfolk populations. *Bulletin of the British Arachnological Society* **9:** 13–18.
Oxford GS. 1993. Genetic drift in the visibly polymorphic spider, *Enoplognatha ovata*—antidote to the peppered moth. *Journal of Biological Education* **27:** 115–120.
Oxford GS, Reillo PR. (in press). The world distribution of species within the *Enoplognatha ovata* group (Araneae: Theridiidae): implications for their evolution and for previous research. *Bulletin of the British Arachnological Society.*
Oxford GS, Shaw MW. 1986. Long-term variation in colour-morph frequencies in the spider *Enoplognatha ovata* (Clerck) (Araneae: Theridiidae): natural selection, migration and intermittent drift. *Biological Journal of Linnean Society* **27:** 225–249.
Reillo PR. 1989. Color polymorphism in the spider *Enoplognatha ovata* (Araneae: Theridiidae): Broad-scale morph-frequency variation in northeastern North America. *American Midland Naturalist* **122:** 199–203.
Reillo PR, Wise DH. 1988a. Genetics of color expression in the spider *Enoplognatha ovata* (Araneae: Theridiidae) from coastal Maine. *American Midland Naturalist* **119:** 318–326.
Reillo PR, Wise DH. 1988b. An experimental evaluation of selection on color morphs of the spider *Enoplognatha ovata* (Araneae: Theridiidae). *Evolution* **42:** 1172–1189.
Reillo PR, Wise DH. 1988c. Temporal and spatial patterns of morph-frequency variation among coastal Maine populations of the polymorphic spider *Enoplognatha ovata* (Araneae: Theridiidae). *American Midland Naturalist* **120:** 337–354.
Snazell R. 1983. On two spiders recently recorded from Britain. *Bulletin of the British Arachnological Society* **6:** 93–98.
Wise DH, Reillo PR. 1985. Frequencies of color morphs in four populations of *Enoplognatha ovata* (Araneae: Theridiidae) in eastern North America. *Psyche* **92:** 135–144.

APPENDIX

Summary of the raw data

Site	set	*E. ovata s.s.*						*E. latimana*				
		L	R	O	S	C	Total	L	R	S	C	Total
1	1	8	1	1	10	3	10	2	0	2	0	2
2	1	1	0	0	1	1	1	5	6	2	5	11
3	1	103	48	2	153	121	153	10	2	2	6	12
4	1	27	11	2	39	32	40	3	3	1	2	6
5	1	66	20	9	94	78	95	11	7	3	9	18
6	1	120	32	15	166	142	167	8	2	2	7	10
7	1	144	42	10	196	185	196	8	1	4	2	9
8	1	8	9	2	19	18	19	—	—	—	—	—
9	1	227	42	6	274	265	275	0	1	0	1	1
10	1	134	36	10	179	168	180	16	6	5	12	22
11	1	102	22	7	131	129	131	3	1	1	2	4
12	1	13	1	0	14	13	14	7	2	3	8	9
13	1	130	43	5	178	172	178	7	0	2	6	7
14	1	118	39	11	167	148	168	1	0	0	0	1
15	1	81	29	15	124	119	125	5	0	0	1	5
16	1	1	1	0	2	1	2	1	0	0	1	1
17	1	134	15	4	151	144	153	—	—	—	—	—
18	1	27	7	4	38	28	38	4	0	0	2	4
19	1	44	6	0	50	49	50	—	—	—	—	—
20	1	114	33	8	152	150	155	—	—	—	—	—
21	1	67	19	1	86	78	87	—	—	—	—	—
22	1	77	31	4	111	93	112	—	—	—	—	—
23	1	104	19	4	121	100	127	—	—	—	—	—
24	1	32	8	1	37	34	41	—	—	—	—	—
25	1	185	19	0	157	170	204	—	—	—	—	—
26	1	152	24	0	153	126	176	1	0	1	0	1
27	1	69	31	7	107	105	107	—	—	—	—	—
28	1	3	1	1	5	3	5	1	0	1	1	1
29	1	29	14	5	48	29	48	14	11	25	14	25
30	1	9	8	2	19	15	19	—	—	—	—	—
31	1	3	1	0	4	2	4	—	—	—	—	—
32	1	33	17	9	59	42	59	—	—	—	—	—
33	2	8	4	2	14	11	14	27	12	39	15	39
34	2	12	7	6	25	19	25	2	1	3	2	3
35	2	36	7	3	46	41	46	25	12	37	25	37
36	2	29	4	0	33	23	33	1	0	1	0	1
37	2	11	11	6	28	27	28	29	19	44	38	48
38	2	—	—	—	—	—	—	2	0	1	1	2
39	2	—	—	—	—	—	—	1	0	0	1	1
40	2	1	0	0	1	1	1	5	4	3	4	9
41	2	29	14	9	52	45	52	0	1	0	1	1
42	2	13	2	1	16	7	16	3	0	2	2	3
43	3	8	3	2	13	13	13	4	4	3	5	8
44	3	5	1	0	6	3	6	—	—	—	—	—
45	3	1	0	1	2	1	2	2	2	4	2	4
46	3	1	2	1	4	2	4	—	—	—	—	—
47	3	18	16	3	37	27	37	1	0	1	1	1
48	3	3	1	2	6	6	6	10	7	6	6	17
49	3	4	2	1	7	4	7	1	0	1	1	1
50	3	0	1	1	2	2	2	1	1	2	1	2
51	3	2	0	0	2	2	2	—	—	—	—	—
52	3	3	0	0	3	2	3	0	1	1	1	1
53	3	1	2	1	4	3	4	5	0	1	5	5
54	3	5	2	3	10	6	10	35	27	18	38	62
55	3	2	2	0	4	4	4	54	24	22	58	78
56	3	14	10	0	24	24	24	43	17	24	54	60

APPENDIX—*continued*

Site	set	*E. ovata s.s.* L	R	O	S	C	Total	*E. latimana* L	R	S	C	Total
57	3	21	4	4	29	26	29	42	21	19	48	63
58	3	45	29	9	83	79	83	23	35	15	42	58
59	3	0	5	0	5	5	5	7	8	6	13	15
60	3	24	8	2	34	33	34	2	3	0	1	5
61	3	4	0	0	4	2	4	—	—	—	—	—
62	3	95	19	18	132	129	132	7	3	5	4	10
63	3	52	14	7	73	72	73	26	32	21	45	58
64	3	29	13	4	46	45	46	12	7	7	18	19
65	3	335	82	21	432	428	438	8	1	3	8	9
66	3	176	45	11	221	222	232	—	—	—	—	—
67	3	162	48	4	207	195	214	—	—	—	—	—

L = No. *lineata* morph, R = No. *redimita* morph, O = No. *ovata* morph S = No. with black spots, C = No. with cocoons.