Geographical distribution of phenotypes regulating pigmentation in the spider *Enoplognatha ovata* (Clerck) (Araneae: Theridiidae)

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The time when red pigment is deposited in the *ovata* and *redimita* morphs of *Enoplognatha ovata* is determined by a closely linked regulatory locus. In Nidderdale, Yorkshire, red pigment in the *ovata* morph is deposited early in development but the *redimita* pattern can develop either early or late. Phenotype frequencies at the regulatory locus define two main areas; one of which has a high and the other a very low frequency of the regulatory phenotype causing early production of red pigment. Phenotype frequencies at the regulatory locus and at the structural locus upon which it acts are not correlated. Bottlenecks in populations in the past might be responsible for allelic distributions at both loci, although selection cannot be excluded.

INTRODUCTION

Variation in regulatory genes in natural populations has received little attention until recently, although the evolutionary importance of changes in gene regulation has long been appreciated in, for example, the evolution of several major groups through paedomorphosis (Gould, 1977). Experiments and observations on regulatory-gene variation (Wilson, 1976; McDonald *et al.*, 1977; McDonald, 1983) together with theoretical analyses (Hedrick and McDonald, 1980) have not yet clarified the relevance, if any, of regulatory-gene variation in extant populations to major phylogenetic events.

Variation at regulatory gene loci can be recognised in three main (but non-exclusive) ways. Regulatory elements may be identified by differences in level of enzyme activity when a structural locus is placed in different genetic backgrounds (e.g. Barnes and Birley, 1978; Tejima and Ohba, 1981; Wilson and McDonald, 1981; Laurie-Ahlberg *et al.*, 1982; Maroni and Laurie-Ahlberg, 1983). Alternatively, different regulatory elements might shift the tissue-specific expression of enzyme activity, or could affect the time when enzymes are produced during development (e.g., Schwartz,

1962; Dickinson, 1975; 1981; Abraham and Doane, 1978; Paigen, 1979; Hoorn and Scharloo, 1981; Allendorf *et al.*, 1982).

Although these studies provide good evidence for the widespread occurrence of regulatory-gene variation little is known of the geographical distribution of different regulatory alleles although such information might suggest selective factors responsible for maintaining them. The little work that has been published concerns shifts in tissue-specific enzyme activity (Powell, 1979) or changes in the timing of gene expression during development (Heller and Volokita, 1981).

Another example of variation in the timing of gene expression is found in the spider *Enoplognatha ovata* (Oxford, 1983). *E. ovata* exhibits three main colour morphs. In *lineata*, the opisthosoma is plain yellow. *Redimita* bears two dorso-lateral carmine stripes across the yellow opisthosoma while in *ovata* the whole of the dorsal surface of the opisthosoma is carmine (see Plate 1a, b, c in Oxford, 1983). These morphs are determined by three alleles at an autosomal locus with a dominance hierarchy such that the *ovata* pattern is top dominant and *lineata* bottom recessive. This hierarchy is always shown by mature females but does not hold in some males (see below).

Figure 1 Map of the Nidderdale study area showing for each site its position relative to roads or tracks (black lines) and the proportion of early developing *redimuta* in total *redimuta* (shaded segment). In sites marked with a dot the null hypothesis, that the frequency of late developing *redimuta* is zero, was not rejected. Marginal numbers indicate Ordnance Survey grid lines. The full map reference for the south western corner is SE164654.

All young spiders have yellow opisthosomas. Red pigment may appear either in the third or fourth instars or at maturity. For patterns which develop early, the frequencies of *redimita* and *ovata* are independent of sex. When pigment is laid down at maturity however, red-patterned morphs are confined to females; males remain yellow (but can be shown to be carrying red-pigment producing alleles). Late developing *ovata* and *redimita* morphs are therefore sex-limited to females. I suggested that this dimorphism in the timing of pigment deposition is controlled by two alleles at a regulatory locus tightly linked to the structural locus. Regulatory alleles are *cis*-acting on their respective structural alleles (Oxford, 1983).

Here I describe the distribution of these two regulatory phenotypes and the three visible morphs in a small area of Nidderdale, Yorkshire.

METHODS

All the *Enoplognatha* sites reported here lie within the area of Nidderdale described by Oxford (1976). The relative locations of the sites are shown in fig. 1 and details of some of their physical attributes and major vegetation components are given in table 1. Sites consist of a patch of broad-leaved

vegetation (often bramble, *Rubus fruticosus* L. *sensu lato* or raspberry, *Rubus idaeus* L.), isolated to a greater or lesser extent from other patches by a stretch of grass or other herbage unsuitable for the construction of nursery retreats by females. Spiders are common within a site and occur only sporadically elsewhere. In two cases (Ja/Jb and E/Q) sites are arbitrary divisions of a continuous stretch of vegetation. Ja and Jb abut one another whereas E and Q, although in the same bramble clump, are separated by a third site (R). All sites border roads or tracks and are backed by dry-stone walls, and each is identified by markers at either end. This is the only habitat in the study area suitable for *E. ovata* since beyond the walls lie pasture or moorland.

Sites were sampled by intensive searching at least twice per season, usually in mid to late June and again in late July to mid August. Each spider was scored for sex, colour pattern phenotype and stage of development (mature/immature). Mature males were recognised by their fully developed palps and mature females by their obvious epigynes. Except on three occasions individuals were released into the ground vegetation after scoring.

On the final sampling occasion in each season only mature females were present and they were found within rolled leaves, often guarding an egg cocoon. These were scored *in situ*.

Table 1 Some physical and vegetational attributes of the Nidderdale sampling sites

Site	Altitude (m)	Aspect	Vegetation
A	262	E.S.E.	Bramble (<i>Rubus fruticosus</i> L. <i>sensu lato</i>) in long grass. Some bracken (<i>Pteridium aquilinum</i> (L.) Kuhn) at north end
B	275	S.S.E.	Bramble with interspersed meadow vetchling (<i>Lathyrus pratensis</i> L.) bordered by long grass
D	239	S.S.E.	Open bramble bordered by long grass
E	278	S.E.	Bramble bordered by long grass
G	267	S.S.E.	Open bramble interspersed with long grass
I	268	S.S.W.	Bramble with willowherb (<i>Chamaenerion angustifolium</i> (L.) Scop.) in long grass
Ja	313	E.S.E.	Hedge woundwort (<i>Stachys sylvatica</i> L.) and scattered raspberry (<i>Rubus idaeus</i> L.) in long grass
Jb	313	E.S.E.	Raspberry and scattered hedge woundwort in long grass
K	299	S.E.	Dense willowherb bordered by long grass
N	328	E.N.E.	Heterogeneous site with bramble, raspberry, bracken and mint (<i>Mentha rotundifolia</i> (L.) Huds.) in long grass
O	270	S.S.W.	Open bramble and nettle (<i>Urtica dioica</i> L.) in long grass
Q	281	S.E.	Bramble bordered by long grass
U	313	E.S.E.	Bramble bordered by long grass
Z	290	S.E.	Heterogeneous site with rose (<i>Rosa canina</i> agg.), dandelion (<i>Taraxicum</i> spp.) and creeping buttercup (<i>Ranunculus repens</i> L.)
AB	318	E.	Scattered nettles in long grass
AC	316	E.S.E.	Scattered hedge woundwort in long grass
AD	309	E.S.E.	Scattered hedge woundwort in long grass
AF	270	N.N.E.	Bramble and scattered hogweed (<i>Heracleum sphondylium</i> L.) in long grass
AO	318	W.	Scattered raspberry in long grass

RESULTS

In Nidderdale *ovata* always develops early, whereas *redimita* may develop either early or late. *Ovata* individuals, therefore, do not provide useful information on regulatory gene variation and are ignored in most calculations. All *redimita* males have an early developing pattern (see introduction). *Redimita* in an immature female must be early developing whereas in mature females this morph may have appeared either early or late. Thus the number of *redimita* in males plus immature females can be used to estimate the frequency of early developing patterns, while those in mature females will yield an estimate of the total frequency of *redimita* (both early and late developing).

For each site in each year numbers of *lineata* and *redimita* in the first sample were compared between males (mature and immature) and immature females using 2×2 contingency chi-squared or Fisher's exact tests. In all cases they were homogeneous. To test whether morph frequency differences between the sexes exist but are not revealed because of small sample sizes, Cochran's Y statistic (Everitt, 1977) was calculated using all 27 comparisons in which at least one *redimita* was scored in the early sample. The value of Y was found to be 1.76, with an associated probability of 0.078. Thus, although there is a tendency for *redimita* frequency to be higher in immature females than in males, this trend is not significant.

Total numbers of *lineata* and *redimita* (males plus immature females) from the first sample were compared with those from the second sample using the same method as above. This comparison tests the null hypothesis that the frequency of late developing *redimita* is zero. The difference in *redimita* frequency between the second and the first samples will estimate the frequency of the late developing pattern.

Confidence limits were placed on the frequency of early developing *redimita* by transforming the estimated frequency to angles ($\sin^{-1} \sqrt{p}$), setting 95 per cent confidence intervals ($\pm 1.96\sqrt{821/N}$, where N = sample size) and then back transforming. Sampling was with replacement in all but three cases, so there will be zero covariance between the first and second estimates of *redimita* frequency. Confidence limits for the difference between *redimita* frequencies in the two samples were therefore based simply on the summation of the variances for each sample, calculated as described above. Finally, the ratio of early developing to

total *redimita* was calculated as the quotient of *redimita* frequencies in the first and in the second samples. The standard error of this ratio is estimated by

$$\sqrt{\frac{\text{Var } p_1 + \frac{p_1^2}{p_2^2} \text{Var } p_2}{p_2^2}}$$

where p_1 and p_2 are the observed frequencies and $\text{Var } p_1$ and $\text{Var } p_2$ are the appropriate binomial variances, $p(1-p)/N$, in the first and second samples, respectively (Kendal and Stuart, 1969 p. 232). The additional term mentioned in Kendal and Stuart (1969) can be ignored because the covariance between samples is zero. Confidence intervals for this ratio cannot be easily calculated since they will be asymmetric to an unknown extent.

The data on which this study is based are given in table 2 and estimates of the frequency of early and late developing *redimita*, and of the ratio of early *redimita* to total, in table 3. The geographical pattern in the value of this ratio is shown in fig. 1. Ratios for all sites are included, irrespective of whether or not they are based on data showing significant differences between first and second samples. This has been done because the two-sample method employed is insensitive to low frequencies of late developing *redimita*.

DISCUSSION

Estimates of phenotype frequencies at the regulatory locus controlling the timing of red pigment deposition in *redimita* were made using a two-sample technique. Although this maintains natural densities of spiders and is quick, the large sampling errors mean that low frequencies of the late developing pattern will not be detected in statistical tests. It would be more efficient to collect spiders early in the season and to rear individual females in the laboratory. However, this requires more effort and perturbs population densities.

Ratios of early developing to total *redimita* were calculated without respect to whether successive samples differed in their proportions of *lineata* and *redimita*. If there really are no late developing *redimita* at a site, then differences in this morph's frequency between first and second samples would result from sampling errors, and should show no systematic bias. However, in pairs of samples taken at sites B, E, G, K, N and Z, 11 of the 13 comparisons have higher *redimita* frequencies in the second sample (binomial test, $P=0.023$), suggesting that some of these colonies

Table 2 Numbers and sex of each visible morph in each collection

Site	Year	Sex	Morph			Total	P†
			<i>lineata</i>	<i>redimita</i>	<i>ovata</i>		
A	82	male	76	4	0	80	P < 0.001
		female I*	98	4	0	102	
		female M*	371	60	0	431	
B	80	male	14	1	0	15	N.S.
		female I	19	2	3	24	
		female M	183	11	28	222	
	81	male	19	0	2	21	N.S.
		female I	23	3	3	29	
		female M	238	23	22	283	
	83	male	48	1	3	52	N.S.
		female I	14	0	1	15	
		female M	669	49	71	789	
	84	male	52	2	8	62	N.S.
		female I	74	6	12	92	
		female M	442	31	31	484	
D	82	male	62	2	0	64	0.05 > P > 0.01
		female I	78	7	0	85	
		female M	101	18	0	119	
	84	male	57	5	0	62	N.S.
		female I	60	3	0	63	
		female M	142	18	0	160	
E	81	male	12	2	4	18	N.S.
		female I	14	2	4	20	
		female M	110	24	17	145	
	84	male	49	5	1	55	N.S.
		female I	95	22	1	118	
		female M	213	47	10	270	
G	82	male	37	5	1	43	N.S.
		female I	76	11	1	88	
		female M	182	30	5	217	
	84	male	35	1	0	36	N.S.
		female I	32	4	0	36	
		female M	49	7	1	57	
I	83	male	20	4	1	25	P = 0.01
		female I	58	9	5	72	
		female M	272	102	26	400	
Ja	80	male	27	0	0	27	P < 0.001
		female I	41	0	0	41	
		female M	105	54	0	159	
Jb	76‡	male	17	0	0	17	P < 0.001
		female I	27	0	0	27	
		female M	370	167	0	537	
	81‡	male	34	1	0	35	P < 0.001
		female I	40	1	0	41	
		female M	296	141	0	437	
	84	male	36	0	0	36	P < 0.001
		female I	60	0	0	60	
		female M	122	59	3	184	
K	81‡	male	30	3	2	35	N.S.
		female I	43	9	3	55	
		female M	260	62	7	329	
	84	male	38	10	0	48	N.S.
		female I	44	4	2	50	
		female M	115	28	2	145	
N	82	male	62	9	0	71	N.S.
		female I	106	14	0	120	
		female M	628	86	0	714	
O	84	male	50	2	1	53	P < 0.001
		female I	88	4	1	93	
		female M	129	34	4	167	

Table 2 (continued)

Site	Year	Sex	Morph			Total	P†
			<i>lineata</i>	<i>redimita</i>	<i>ovata</i>		
Q	81	male	27	9	2	38	N.S.
		female I	29	4	0	33	
		female M	77	23	0	100	
	84	male	36	3	3	42	0.01 > P > 0.001
		female I	62	13	1	76	
U	81	female M	240	84	8	332	P < 0.001
		male	19	0	2	21	
		female I	53	2	1	56	
	84	female M	137	68	11	216	P < 0.001
		male	27	0	0	27	
Z	81	female I	66	2	3	71	P < 0.001
		female M	62	25	4	91	
		male	14	2	0	16	
	84	female I	17	10	0	27	N.S.
		female M	46	21	0	67	
AB	83	male	6	4	0	10	N.S.
		female I	13	6	0	19	
		female M	20	14	0	34	
AC	82	male	46	0	0	46	0.01 > P > 0.001
		female I	69	1	0	70	
		female M	299	30	0	329	
AD	81	male	86	0	0	86	P < 0.001
		female I	89	0	3	92	
		female M	420	119	17	556	
AF	81	male	43	3	0	46	0.01 > P > 0.001
		female I	62	4	0	66	
		female M	119	31	0	150	
	84	male	70	3	0	73	0.01 > P > 0.001
		female I	83	10	0	93	
AO	83	female M	241	54	2	297	P < 0.001
		male	38	0	0	38	
		female I	86	1	0	87	
AO	83	female M	261	69	0	330	0.01 > P > 0.001
		male	22	0	0	22	
		female I	84	0	0	84	
		female M	249	23	0	272	

* I = Immature, M = Mature.

† Probability associated with a 2 × 2 chi-squared test for differences in numbers of *lineata* and *redimita* between female_I + male and female_M.

‡ First samples collected from the field and reared in the laboratory. Changes in females on reaching maturity are as follows (*lineata/redimita*): Jb ('76) 27/0 to 21/6; Jb ('81) 40/1 to 26/15; K ('81) 43/9 to 42/10.

might have low frequencies of late developing *redimita*. Indeed, there is direct evidence for this at site K. In a small sample of immature individuals returned to the laboratory for breeding purposes one *lineata* female changed to *redimita* on reaching maturity (see footnote to table 2).

Two main regions are defined by the distribution of regulatory phenotypes (fig. 1). The south/central area (sites G, B, E, Q, Z and K) has a high frequency of the early developing phenotype which decreases to the north and south. This phenotype is essentially absent from the region above site AD. The low frequencies in U and Jb almost certainly result from an experimental

introduction from a site between B and E (Oxford and Shaw, in preparation).

One anomaly is the isolated site N in the extreme north of the area, where all *redimita* apparently develop early. In 1975 a small sample (N = 50) revealed only *lineata* but one year (one generation) later, one *redimita* was found in a sample of 93 individuals. Since that time, the frequency of *redimita* has increased and in 1984 had reached a value of 0.134. The large mean population size at this site, estimated from the number of mature females sampled, means that the increase can only be ascribed to migration or selection (Oxford and Shaw, in prep.). The lower

Table 3 Summary statistics of phenotype frequencies at the regulatory locus

Site	Frequency in mature females		Estimated frequency of <i>redimita</i> (in <i>redimita</i> + <i>lineata</i>)					fE/fT.†	S.E.
	<i>redimita</i>	<i>ovata</i>	Early developing	95 per cent C.I.*	Late developing	95 per cent C.I.*			
A	0.139	0	0.044	0.019–0.078	0.095	0.051–0.152	0.316	0.116	
B	0.064	0.085	0.054	0.031–0.083	0.016	—	0.770		
D	0.129	0	0.062	0.037–0.094	0.067	0.031–0.114	0.481	0.135	
E	0.171	0.065	0.154	0.108–0.207	0.026	—	0.856		
G	0.135	0.022	0.104	0.066–0.150	0.033	—	0.757		
I	0.255	0.065	0.143	0.079–0.222	0.130	0.063–0.216	0.524	0.142	
Ja	0.340	0	0	—	0.340	0.269–0.415	0		
Jb	0.317	0.003	0.009	0.001–0.026	0.308	0.244–0.377	0.029	0.021	
K	0.190	0.019	0.144	0.096–0.198	0.050	—	0.742		
N	0.120	0	0.120	0.078–0.170	0	—	1.000		
O	0.204	0.024	0.042	0.015–0.080	0.167	0.092–0.258	0.200	0.085	
Q	0.248	0.018	0.158	0.109–0.215	0.094	0.050–0.150	0.628	0.119	
U	0.303	0.049	0.024	0.006–0.052	0.295	0.213–0.384	0.074	0.037	
Z	0.347	0	0.306	0.205–0.416	0.041	—	0.882		
AB	0.092	0	0.009	4×10^{-6} –0.033	0.083	0.034–0.150	0.094	0.096	
AC	0.214	0.031	0	—	0.221	0.187–0.257	0		
AD	0.190	0.004	0.072	0.045–0.105	0.119	0.075–0.172	0.377	0.089	
AF	0.209	0	0.008	4×10^{-6} –0.031	0.201	0.125–0.289	0.038	0.038	
AO	0.085	0	0	—	0.085	0.054–0.120	0		

* 95 per cent confidence interval. A—in column 7 indicates the null hypothesis, that the frequency of late developing *redimita* is zero, was not rejected. † fE and fT refer to the frequencies of early developing and total *redimita* respectively.

overall frequency of *redimita* in the nearest colonies to N, AB and AO, and the striking differences at the regulatory locus reduces the probability that migration is an explanation.

In populations fixed for one or the other allele, the regulatory locus determines when the population becomes visibly polymorphic (given that the frequency of *ovata* is low in virtually all sites nearly fixed for the late allele). In 1980 sites B and Ja were sampled at two week intervals from May 21st onwards. Carapace widths of all individuals were measured and approximate instars assigned with the tables in Seligy (1971). Most spiders were in their third instar on May 21st and the majority were fourth instar on June 4th. By June 4th, the proportions of morphs at site B were statistically indistinguishable from those in the sample of mature females scored in August. At Ja all spiders were *lineata* although carapace size distributions were identical to those at B on this and all other sampling dates. Not until July 1st was the first *redimita* found at Ja and by July 18th 29.7 per cent of females were of this morph.

Thus, populations in sites B and Ja differ by between four and six weeks in developing a visible polymorphism. As soon as females are mature they mate and one to two weeks later have established themselves in rolled leaves prior to egg laying. At late developing sites, therefore, the spider population is visibly polymorphic and exposed to selective

factors e.g. predators or solar radiation, for only two weeks, so that if visible, rather than pleiotropic, effects of the structural alleles are of importance, selection must act swiftly. As yet there is no direct evidence for selection on the visible morphs.

Although the areas of approximately uniform phenotype frequencies at the regulatory locus are small in terms of absolute distances, they are large compared to the between-site variability found for the visible morphs (Oxford, 1976; Oxford and Shaw, in prep.). The extreme differentiation at these two loci might result from bottlenecks in the past. In the late 1940's the main roads through the area were widened and resurfaced and many of the existing verges were disturbed by excavation (B. Gill, personal communication). If small isolated populations of spiders survived, then stochastic events, both at and immediately after their formation, may have perturbed allele frequencies at both loci. The present situation might represent a stage in the gradual return to a global equilibrium for the area, mediated by migration and/or selection (Oxford and Shaw, in prep.). None of the correlations between the ratio of early developing to total *redimita* and the frequencies in mature females of *lineata*, *redimita* and *ovata* (table 3) are significant, and there are no obvious associations between this ratio and altitude, aspect or vegetation (table 1). Observed changes at site N since 1975 suggest that selection, either on the

structural locus itself or on closely linked loci, does play a role in determining morph frequencies.

As far as I am aware only one other published study has compared the distribution of genetic variation at associated regulatory and structural loci within a species. Powell (1979) investigated three polymorphisms affecting the amylase (*Amy*) locus in *Drosophila pseudoobscura*. He found coordinated variation of electrophoretically distinct alleles at the *Amy* locus and chromosome 3 inversions but no association between these and patterns of expression of *Amy* in the midgut, controlled by regulatory elements (Powell and Lichtenfels, 1979). An independence of midgut patterns and electrophoretic variation is also found in comparisons of seven other *Drosophila* species (Powell *et al.*, 1980). A similar lack of correlation exists between variation at regulatory and structural loci in *Enoplognatha*.

Heller and Volokita (1981) found that geographical differences in the timing of black pigment deposition in the shell of *Xeropicta vestalis* in Israel were of adaptive significance. Shell pigmentation is important in the thermal adjustment of the snail and the initiation and cessation of pigment formation was found to be appropriate to the contrasting climates of mountain and coastal plain. Powell and Andjelković (1983) and Powell and Amanto (1984) found directional changes in the frequencies of midgut amylase patterns in *Drosophila pseudoobscura* under stress conditions. In populations living on medium containing starch as the only carbohydrate source, one midgut pattern increased in frequency. No such increase was observed in control cages with medium containing maltose as the sole carbohydrate source. Powell and co-workers argue that these changes are best interpreted as the result of selection acting on the regulatory loci controlling midgut patterns. As yet, no adaptive function can be ascribed to geographical variation at the regulatory locus in *Enoplognatha*, although one cannot discount the possibility that one exists.

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