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Genetic differentiation and natural hybridization between two morphological forms of the common woodlouse, *Oniscus asellus* Linnaeus 1758

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The common woodlouse *Oniscus asellus* can be divided into two forms on the basis of morphology, particularly male accessory genitalia. Where these taxa meet, morphological intermediates are found, and the forms were therefore described as subspecies; *O. a. asellus* and *O. a. occidentalis*. In this study allozyme loci are used to test the hypothesis that intermediate forms result from hybridization, and to study the nature of hybridization. Thirteen enzyme loci were scored across five English sites representative of each subspecies and intermediates. Ten loci showed strong frequency differences between *asellus* and *occidentalis* populations, although no loci showed completely fixed differences. These data confirm that *asellus* and *occidentalis* represent genetically distinct taxa, and that intermediate populations are of hybrid origin. There is apparently substantial population substructuring in the contact zone, as indicated by deficits of heterozygotes (F_{IS}) and sporadic gametic (i.e. linkage) disequilibria. Population structure in the *Oniscus* hybrid zone appears to be analogous to that seen in plant hybrid swarms rather than the narrow hybrid zones observed in many animal taxa. Values of Nei's genetic distance between the subspecies range from 0.65 to 0.70; these are much higher than between typical conspecific taxa and are indicative of ancient genetic divergence. However, because *asellus* and *occidentalis* do not remain distinct in areas of overlap, it is simplest to regard these taxa as members of the same species.

Keywords: allozymes, hybrid zone, linkage disequilibrium, *Oniscus*, subspecies.

Introduction

The woodlouse *Oniscus asellus* Linnaeus is one of the most widespread and abundant terrestrial arthropods in Western Europe. Bilton (1994) demonstrated that the species could be divided into two distinct subspecies: *Oniscus asellus asellus* Linnaeus and *O. asellus occidentalis* Bilton. The two taxa are ecologically and biogeographically distinct as well as differing in morphology. True *asellus* is widespread, particularly in synanthropic (human-influenced) sites. On the other hand, *occidentalis* is recorded only from sites in the south-west of the British Isles and western France (Bilton, 1994, 1997). Intermediate populations occur throughout the range of *occidentalis*, and also further east, where they are increasingly confined to wet habitats. Bilton (1994) suggested that the numerous populations with intermediate morphology arose via hybridization between the two subspecific taxa. This paper presents the results of an allozyme study of five populations of *Oniscus asellus s. lat.* to test the hypothesis that hybridization explains the intermediate morphologies found, and to study the nature of the hybridization. We provide genetic evidence of strong divergence between the taxa and discuss the status of these forms.

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Materials and methods

Site selection and specimen collection

Oniscus specimens were collected from three sites in Devon (Wistman's Wood [ancient *Quercus petraea* woodland; *occidentalis*], Lydford Gorge [managed mixed woodland; intermediates] and Braunton [village garden;

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intermediates]) together with sites in Northumberland (Riding Woods [seminatural *Quercus robur* wood; *asellus*]) and London (Highbury [urban garden; *asellus*]).

At each locality *Oniscus* specimens were collected by hand searching in humid shelter sites such as beneath moss and bark on rotting logs. Only males were selected because female *occidentalis* and intermediate morphs are not reliably separable from *asellus* (Bilton, 1994). Woodlice were brought alive to the laboratory, where they were scored as *asellus*, *occidentalis* or intermediates on the basis of male genitalia, before being transferred directly to a −70°C freezer for storage prior to allozyme studies.

Allozyme electrophoresis

For allozyme studies the head and pereon (leg-bearing segments) of each individual were removed, and the pleon (hind body) stored in ethanol for further study of the accessory genitalia. Each pereon was homogenized in 100 μL of grinding buffer (0.1 M Tris/HCl pH 8.0; five drops of β-mercaptoethanol/100 mL). The homogenate was then centrifuged for 5 min at 10 000 ***g*** in a microcentrifuge and stored on ice.

Electrophoresis and enzyme staining

A total of 23 enzyme systems were initially screened. Of these, 12 (aconitase, adenosine deaminase, adenylate kinase, esterase, fumarase, glucose-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, α-glucose phosphate dehydrogenase, glutathione reductase, hexokinase, isocitrate dehydrogenase and nucleoside phosphorylase) showed no activity or were unscorable, leaving 11 enzyme systems with a total of 13 scorable loci (acid phosphatase, *Acp*; enolase, *Eno*; glutamic-oxaloacetic transaminase, *Got-1*, *Got-2*; glucose-6-phosphate isomerase, *Gpi*; malate dehydrogenase, *Mdh*; malic enzyme, *Me*; mannose-6-phosphate isomerase, *Mpi*; peptidase (leucine glycine glycine), *Lgg-1*, *Lgg-2*; peptidase (phenylalanine proline) *Pp*; phosphoglucomutase, *Pgm*; and sorbitol dehydrogenase, *Sdh*). The running conditions on cellulose acetate gels were: *Acp*, *Eno*, *Got-1*, *Got-2* and *Mdh*, phosphate buffer, 20 min at 200 V; *Pp*, Tris-glycine, 15 min at 200 V; all other enzymes, Tris-glycine, 20 min at 200 V. Buffers were as follows: Phosphate buffer, 20 mM NaH_2PO_4, 6 mM Na_2HPO_4, pH 6.3; Tris-glycine, 20 mM Tris base, 200 mM glycine, pH 8.0. Enzyme staining followed Easteal & Boussy (1987), Emelianov *et al*. (1995) and Richardson *et al*. (1986).

Data analysis

The following were calculated for each of the five localities: mean number of alleles per locus, percentage of loci polymorphic (95 and 99% criteria) and mean heterozygosity. Population structure and deviations from Hardy–Weinberg equilibrium were assessed using *F*-statistics (Wright, 1965, 1978).

To display the genotypic structure within the hybrid zone, a 'hybrid index' was constructed based on allelic differentiation between the two taxa. Alleles at each of the 10 differentiated loci were classed as typical of either *asellus* or *occidentalis*. The hybrid index value of an individual represents the proportion of *asellus* alleles that it carries, and varies between 0 and 1.

To assess population structure and gene flow between the two taxa further, pairwise linkage disequilibria (gametic correlations) were estimated for each population between each of the 10 differentiated loci. Because we were interested in gene flow between taxa, we lumped alleles at each locus if they belonged to the same taxon. Thus each locus was treated as if it had only two alleles: *O* (*occidentalis*) and *A* (*asellus*). With 10 loci, this procedure also reduces the number of pairwise disequilibria to be estimated to 45 per site. Maximum likelihood pairwise linkage disequilibria were calculated and their significance tested following Hill (1974). We estimated the correlation coefficient R_{ij} to within an accuracy of 0.01 for each pair of loci i and j. The correlation coefficient is calculated because it varies between 0 and 1, allowing a comparison of disequilibria between loci of different frequencies. The correlation coefficient is a standardization of the disequilibrium coefficient, D_{ij}, as follows:

$$R_{ij} = \frac{D_{ij}}{\sqrt{p_i(1-p_i)p_j(1-p_j)}}.$$

The correlation coefficient has been criticized as a measure of linkage disequilibrium on the grounds that it is dependent on the allele frequencies p_i and p_j (Hedrick, 1987). However, the alternative standardization recommended by Hedrick (Lewontin's D_{ij}*) has its own problems: although its limits are not frequency-dependent, its value is frequency-dependent; D_{ij}* is also statistically intractable because it is a discontinuous function (Lewontin, 1988). There is no easy solution to this problem of standardization, which is useful to compare disequilibria across loci which differ in p_i and p_j. We here adopt the correlation coefficient which is at least familiar from other statistical problems. Hill's likelihood method for estimating the within-gamete disequilibrium, D_{ij}, implicitly makes the assumption that the between-gamete disequilibrium, $D_{i/j}$, is zero (Weir, 1990). This assumption seems reasonable provided that $F_{IS} = 0$ (F_{IS} is essentially a within-locus between-gamete disequilibrium). Unfortunately, we find

here that F_{IS} is frequently nonzero, although the reasons for this may be in some cases more to do with scoring difficulties or possible hemizygosity of sex-linked loci than true absence of heterozygotes. Because the gametic arrangements of double heterozygotes cannot be detected from allozyme data, our estimate of D_{ij} is equivalent to Weir's Δij $= D_{ij} + D_{i/j}$.

Nei's unbiased genetic identity, I, and genetic distance, D_N (Nei, 1972, 1986) were calculated between populations. Genetic identities were then clustered using the neighbour-joining technique, correcting for negative branch lengths (negative branch lengths set to zero; Felsenstein, 1993; Swofford *et al*., 1997). Calculations were carried out using BIOSYS-1 (Swofford & Selander, 1989), except for calculations of disequilibria, which were performed using a computer program written by J. M., and the neighbour-joining tree construction which was performed using PHYLIP version 3.5c (Felsenstein, 1993).

Results

Morphological scoring

The population from Wistman's Wood consisted of pure *O. asellus occidentalis*, based on male accessory genital morphology (Bilton, 1994). Using the same criteria the populations from North London and Northumberland were *O. asellus asellus*, which is the only form known from either of these regions (Bilton, 1994). The Braunton sample consisted of intermediate individuals. Specimens from Lydford were of mixed morphology; 33 specimens approximated *O. asellus asellus* on male genital morphology, although they also had some narrowing of body form and pale pereon markings characteristic of *occidentalis* and intermediate populations, suggesting that these individuals too were of mixed origin (Bilton, 1994). Three additional specimens from Lydford could be classified as *occidentalis* on male characteristics. Overall the population was considered to be intermediate in character between *asellus* and *occidentalis*.

Allele frequencies

Table 1 presents allele frequencies at scorable loci for the five populations investigated. All 13 loci show some degree of variation within and between populations. At 10 loci (*Eno*, *Got-1*, *Got-2*, *Lgg-1*, *Lgg-2*, *Mdh*, *Me*, *Mpi*, *Pgm* and *Pp*) there were >30% frequency differences at at least one allele between the *asellus* and *occidentalis* samples. Thirty-one alleles at 10 semidiagnostic loci were used to calculate a 'hybrid index' for individuals within each population (Fig. 1), which clearly characterizes the morphologically intermediate Braunton and Lydford populations as genetically intermediate. These also had allele frequencies at diagnostic loci intermediate between those of Wistman's Wood (*occidentalis*) or London/Northumberland (*asellus*; see Fig. 1). In addition, individuals from mixed populations were themselves genetically intermediate, rather than bimodal with individuals genetically similar to one or other parent.

Levels of genetic variability within populations

All three measures of genetic variability (Table 2) are lower in *O. asellus asellus* populations (London and Northumberland) than in those consisting of *occidentalis* (Wistman's) or intermediates (Lydford and Braunton). Heterozygosity in intermediate populations was about double that in pure populations.

Population structure

Values of F_{IS} in Table 3 indicate significant deviations from Hardy–Weinberg equilibrium at a number of loci, all of these being the result of heterozygote deficiencies ($F_{IS} > 0$). *Me* shows a highly significant heterozygote deficiency in all populations, which may, however, result from scoring inaccuracies, sex linkage or null alleles, as no heterozygotes were scored ($F_{IS} = 1.00$), even from polymorphic populations; the difficulties were compounded because homozygotes were themselves double-banded on the cellulose acetate plates. Although results for this locus must be treated with caution, *Me* is included here because it could still have proved informative for linkage disequilibrium, and genetic differentiation was observed between the populations studied. *Mpi* and *Pgm* also had high values of F_{IS} in most populations. The sporadic pattern observed across the other loci could result from inbreeding or from the pooling of subpopulations which differ in allele frequency.

Gametic disequilibria

Gene flow between genetically differentiated populations at selection/gene flow equilibrium is liable to produce strong gametic phase disequilibria. Such disequilibria can be used to measure gene flow between populations. We therefore tested for pairwise disequilibrium amongst the 10 differentiated loci, using the alleles recoded according to subspecies of origin (Table 1). Thus, at each differentiated locus, all *occidentalis* alleles were lumped, and all *asellus* alleles were lumped. If significant levels of recent gene flow had been occurring, we would expect that significant levels of positive R would be found in the morpho-

logically and genetically intermediate populations at Lydford Gorge and Braunton. Instead, the average value of the disequilibrium coefficient R was low across all populations, and not significantly different from 0 (Table 3). Nor is there an obvious pattern of significant disequilibria across loci (Table 4). The two intermediate populations (Lydford, Braunton) do have marginally more significant disequilibria, six and nine out of 45, respectively, than pure *occidentalis* (Wistman's: two significant disequilibria) and pure *asellus*

Table 1 Summary of allele frequencies at all loci in *Oniscus asellus s. lat*. (and see next page)

			Population				
Locus*	Allele	Allele in hybrid index†‡	Wistman's Wood	Lydford Gorge	Braunton	London	Northumberland
Acp	(*N*)		59	34	30	31	36
	A	–	0.008	0.015	0.050	0.000	0.000
	B	–	0.992	0.985	0.933	1.00	0.972
	C	–	0.000	0.000	0.017	0.000	0.028
*Eno**	(*N*)		75	35	32	32	36
	A	A	0.073	0.614	0.219	0.969	1.00
	B	O	0.927	0.386	0.781	0.031	0.000
*Got-1**	(*N*)		75	36	32	32	36
	A	A	0.100	0.778	0.672	0.844	0.972
	B	O	0.713	0.153	0.281	0.156	0.028
	C	O	0.187	0.069	0.047	0.000	0.000
*Got-2**	(*N*)		63	36	32	32	26
	A	A	0.071	0.750	0.188	0.938	1.00
	B	O	0.929	0.250	0.813	0.063	0.000
Gpi	(*N*)		75	35	32	32	36
	A	–	0.033	0.186	0.078	0.047	0.000
	B	–	0.967	0.814	0.922	0.953	1.00
*Lgg-1**	(*N*)		72	35	31	31	35
	A	O	0.868	0.257	0.532	0.016	0.000
	B	A	0.132	0.743	0.468	0.968	0.857
	C	A	0.000	0.000	0.000	0.016	0.129
	D	A	0.000	0.000	0.000	0.000	0.014
*Lgg-2**	(*N*)		69	35	32	31	36
	A	O	0.000	0.014	0.000	0.000	0.000
	B	O	0.768	0.514	0.656	0.532	0.431
	C	A	0.232	0.471	0.328	0.468	0.569
	D	(A)	0.000	0.000	0.016	0.000	0.000
*Mdh**	(*N*)		72	33	32	32	35
	A	A	0.007	0.515	0.281	0.859	0.386
	B	(O)	0.993	0.485	0.719	0.141	0.557
	C	A	0.000	0.000	0.000	0.000	0.057
*Me**	(*N*)		62	26	18	20	36
	A	O	0.968	0.192	0.333	0.050	0.000
	B	A	0.032	0.769	0.611	0.950	1.00
	C	(O)	0.000	0.038	0.056	0.000	0.000
*Mpi**	(*N*)		73	34	32	32	36
	A	O	0.048	0.000	0.016	0.000	0.000
	B	A	0.281	0.838	0.781	0.938	1.00
	C	O	0.671	0.162	0.203	0.063	0.000
*Pgm**	(*N*)		75	34	32	29	36
	A	O	0.640	0.368	0.172	0.000	0.167
	B	A	0.313	0.632	0.828	0.983	0.833
	C	A	0.047	0.000	0.000	0.000	0.000
	D	O	0.000	0.000	0.000	0.017	0.000

Table 1 Cont.

Locus*	Allele	Allele in hybrid index†‡	Population				
			Wistman's Wood	Lydford Gorge	Braunton	London	Northumberland
*Pp**	(*N*)		68	33	32	27	35
	A	O	0.978	0.712	0.531	0.796	0.271
	B	A	0.022	0.258	0.438	0.148	0.600
	C	A	0.000	0.030	0.031	0.056	0.129
Sdh	(*N*)		75	35	32	32	36
	A	–	0.000	0.000	0.016	0.000	0.000
	B	–	1.00	1.00	0.984	1.00	1.00

*Used in hybrid index.
†O, *occidentalis*; A, *asellus*.
‡Alleles whose subspecific designation is within parentheses are poorly differentiated and were assigned to the more appropriate taxon. Alleles present only in intermediate populations were assigned to either *asellus* or *occidentalis*.

(London: one; Northumberland: one). However, some significant values of R are negative, unexpected under deterministic gene flow, suggesting that genetic drift resulting from patchy population structure caused most of the sporadically significant disequilibria, rather than gene flow across a strongly selected hybrid zone.

Inter-relationships between populations

Values of Nei's unbiased genetic identity and distance both highlighted the distinction between the Wistman's Wood *occidentalis* populations and *asellus*, the Wistman's sample having a mean identity of 0.509 when compared with *asellus*. Mean identity between Wistman's and intermediate populations was 0.777, identity between the two *asellus* populations being 0.955. Values of genetic distance ranged from 0.700 (Wistman's Wood vs. Northumberland) to 0.042 (Lydford Gorge vs. London). The neighbour-joining phenogram of genetic identity, after correction for negative branch lengths, is shown in Fig. 2. The phenogram clusters the London and Northumberland populations (both *asellus*), and places the Wistman's *occidentalis* population at the other end of the network, with the hybrid populations (Lydford Gorge and Braunton) falling between the two extremes.

Discussion

The principal aim of the present study was to discover whether patterns of morphological differentiation within *Oniscus asellus* were supported by genetic data, and to investigate whether morphologically intermediate populations resulted from hybridization.

Bilton (1994) interpreted populations of intermediate morphology as arising through hybridization between

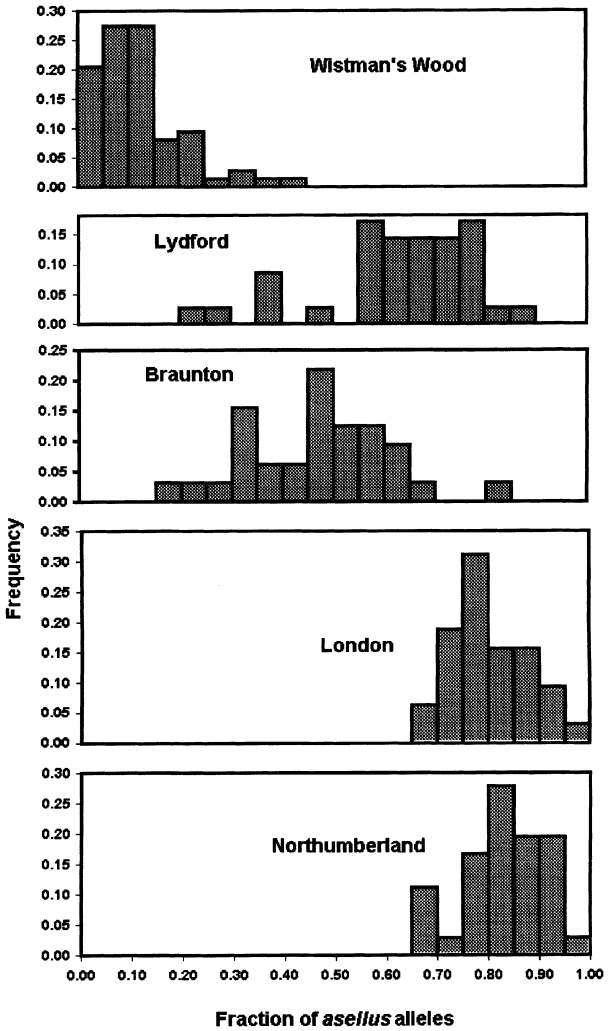

Fig. 1 Frequency distribution of *asellus* alleles at semidiagnostic loci for individuals in the five populations of *Oniscus asellus s. lat.* investigated.

Table 2 Measures of genetic variability within each population of *Oniscus asellus s. lat.*

Population	Mean no. alleles/locus	Loci polymorphic* (%)	Mean heterozygosity/ locus†
Wistman's Wood	2.15 (0.15)	53.9/76.9	0.190 (0.052)
Lydford Gorge	2.23 (0.17)	84.6/92.3	0.349 (0.046)
Braunton	2.46 (0.14)	92.3/100	0.348 (0.045)
London	2.00 (0.16)	53.9/84.6	0.150 (0.042)
Northumberland	1.77 (0.23)	38.5/53.9	0.173 (0.063)

Standard errors are given in parentheses.
*95/99% levels, respectively.
†Unbiased expected estimate (assumes Hardy–Weinberg equilibrium).

asellus and *occidentalis* subspecies. The genetic data presented here support this conclusion. Ten of the 13 loci scored have alternate alleles at high frequency in *occidentalis* and *asellus* populations, although none of these is entirely diagnostic. Allele frequency data (Table 1) confirm that the Braunton and Lydford populations contain the most even mixture of semidiagnostic alleles; this is also clearly displayed in the hybrid index scores (Fig. 1). Braunton and Lydford have the highest levels of genetic variability, reflecting probable mutual introgression of *asellus* and *occidentalis* genotypes.

Values of Nei's genetic distance between *occidentalis* and *asellus* populations (0.65–0.70) fall well above those expected of conspecific taxa, which are typically less than 0.15 (Avise & Selander, 1972; Selander & Johnson, 1973; Ayala *et al*., 1974; Emelianov *et al*., 1995). Although this strongly suggests that the taxa have had a long period of somewhat independent evolution affecting the majority of genetic loci, interpreting them as separate species would require a purely distance-based concept. Under the more traditional Biological Species Concept (Mayr, 1963), these taxa are clearly members of a single interbreeding community where they come in contact, and they would be classed as conspecific. There is also no evidence that the taxa form separate species under the genotypic cluster definition (Mallet, 1995); species defined via genotypic clusters should retain their integrity, and intermediates remain in a minority compared to parental forms in zones of overlap. This is not the case with *Oniscus asellus*, where intermediates are frequent in mixed populations in nature. Indeed pure *occidentalis* populations are rare, and in spite of the great genetic distance between *asellus* and *occidentalis*, the two forms are best considered as subspecies.

Over a wide area of southern England and western France intermediate *Oniscus* populations dominate most seminatural habitats. Rather than hybridization occurring in a narrow tension zone, the interaction

Table 3 Heterozygote deficit (F_{IS}) values by locus, and average gametic phase disequilibrium (R) between lumped alleles in each population of *Oniscus asellus s. lat.*

Locus	Number of alleles	F_{IS}				
		Wistman's Wood	Lydford Gorge	Braunton	London	Northumberland
Acp	3	−0.009	−0.015	−0.057	–	−0.029
Eno	2	0.313**	0.578**	0.269	−0.032	–
Got-1	3	0.134	0.011	0.331	0.526**	1.000***
Got-2	2	0.402**	0.111	−0.026	−0.670	–
Gpi	2	−0.340	−0.039	−0.039	0.049	–
Lgg-1	4	0.212	0.252	−0.101	−0.025	0.080
Lgg-2	4	0.186	0.332	0.187	0.158	0.264
Mdh	3	−0.007	0.272	0.536**	0.612**	0.203*
Me	3	1.000***	1.000***	1.000***	1.000***	–
Mpi	3	0.444***	0.675***	0.372*	0.670	–
Pgm	4	0.265***	0.684***	0.431*	−0.018	0.800***
Pp	3	−0.023	0.146	0.524**	−0.195	0.013
Sdh	2	–	–	−0.016	–	–
	Mean F_{IS}	0.215	0.341	0.260	0.180	0.333
	Mean R	0.02	0.08	0.04	−0.03	0.01
	(SE, $n = 45$)	(0.02)	(0.03)	(0.03)	(0.02)	(0.01)

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 4 Linkage disequilibria for populations of *Oniscus asellus s. lat.*

	Got-1	*Got-2*	*Lgg-1*	*Lgg-2*	*Mdh*	*Me*	*Mpi*	*Pgm*	*Pp*
Eno	ooooo	ooooo	oo+oo	oo+oo	oo+oo	ooooo	+oooo	oo+oo	ooooo
Got-1		ooooo	ooooo	ooooo	ooooo	oo+oo	ooo+o	ooooo	ooooo
Got-2			ooooo	ooooo	ooooo	ooooo	+++oo	ooooo	ooooo
Lgg-1				ooooo	ooooo	ooooo	ooooo	o+ooo	ooooo
Lgg-2					ooooo	o+ooo	ooooo	ooooo	ooooo
Mdh						oo−oo	ooooo	ooooo	o− −oo
Me							o+ooo	o+ooo	oo+oo
Mpi								ooooo	ooooo
Pgm									oooo+

Significant positive (+), negative (−) and nonsignificant ($G_1 < 3.84$, o) gametic correlations are shown between all pairs of loci. The string of five symbols represents results of tests at each site. Order of sites: Wistman's Wood, Lydford, Braunton, London, Northumberland.

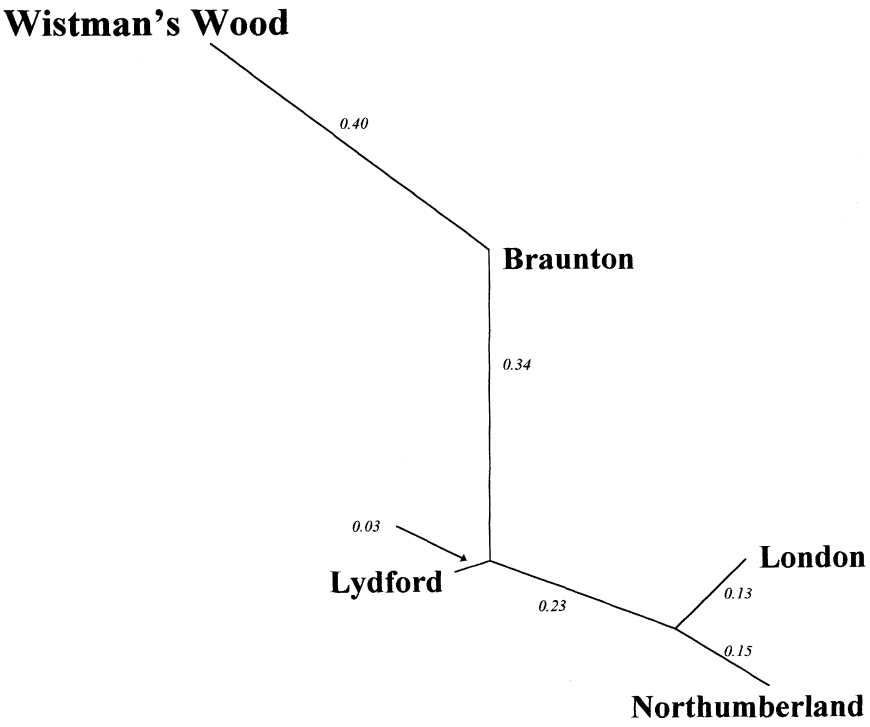

Fig. 2 Neighbour-joining phenogram (corrected for negative branch lengths) of Nei's unbiased genetic identity between the five populations of *Oniscus asellus s. lat.* sampled in the current study. Values in italics represent branch lengths.

between the two *asellus* subspecies appears to be most analogous to that seen in a number of plant taxa (*see* Arnold, 1997) where hybridization results in a mosaic of populations of parents and hybrids, the exact location of which may be mediated by stochastic population dynamics and habitat. Stochastic colonization of habitat patches by woodlice appears to result in sporadic significant heterozygote deficits and linkage disequilibria locally. The lack of significant positive disequilibria between most pairs of loci suggests that gene flow is weak compared with the width of the hybrid belts (Barton & Gale, 1993). Further genetic investigations of *Oniscus* populations along local transects would now be useful to test the ideas generated in this study.

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