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Patterns of morphological and genetic variability in UK populations of the shore crab,
Carcinus maenas Linnaeus, 1758 (Crustacea: Decapoda: Brachyura)

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ABSTRACT

Previous research has identified extensive inter-population variability in the morphology of the shore crab (Carcinus maenas L.). To determine the source of this variation (genetic or environmental), morphological and genetic data were analysed from crabs collected from eight sites around the coast of the UK. Ten morphometric traits were measured from over 800 crabs and the degree of morphological similarity among sites calculated using multivariate techniques. Allozyme electrophoresis was used to investigate patterns of genetic similarity. Extensive morphological variability was detected; eight out of the ten morphometric traits analysed were useful when discriminating between crabs from each site. Discriminant function analysis revealed that over 35% of individuals could be classified to their site of origin on the basis of their morphology. In contrast, the allozyme analysis revealed low levels of genetic variability, both within the meta-population and among the crab population at each site. Pairwise comparisons revealed a moderate correlation between the degree of morphological and genetic similarity of crabs at each site, which suggests that the observed phenotypic variability has a genetic component. However, only around 20% of the phenotypic variability detected was associated with the patterns of genetic similarity. This means that patterns of morphological variability in this species are largely determined by the local environmental conditions: local factors could have a within-generation selective influence on mean trait values or C. maenas may exhibit phenotypic plasticity.

Key words: Carcinus maenas, genetic variation, morphology, phenotypic plasticity, shore crab.

INTRODUCTION

Until recently, the analysis of inter-population variability in the morphology of the shore crab, Carcinus maenas, has focused on the longstanding debate regarding the classification of this species in European waters (Yamada and Hauck, 2001). A number of studies have provided evidence that variations in male pleopod structure and carapace length to width ratio, combined with differences in the distribution of setae on the front distal region of the cheliped carpus, supports the separation of shore crab specimens from Atlantic and Mediterranean regions into two distinct species: C. maenas (Linnaeus, 1958) and C. aestuari (Nardo, 1847) (Demeusy and Veillet, 1953; Zariquie Alvarez, 1968; Yamada and Hauck, 2001). In contrast, other research has demonstrated that there is a degree of overlap between the morphological features of crabs from each region, which means that an individual cannot be unequivocally assigned to the Atlantic or Mediterranean group on the basis of shape (e.g. Clark et al., 2001). The controversy regarding the taxonomy of this species is ongoing and shore crab specimens are still classified by locality as opposed to any biological basis.

The classification of Atlantic and Mediterranean shore crabs on the basis of their genetic population structure is similarly controversial. Patterns of allozyme variation revealed a lack of complete separation among regional populations (Bulheim and Bahns, 1996), whereas gene-sequencing studies have detected significant divergence. For example, Geller et al. (1997) assigned C. maenas and C. aestuari the status of sibling species on the basis of their genetic population structure and Roman and Palumbi (2004) identified a clear break at the species level. The ambiguity concerning the classification of this species is likely to have arisen as a result of its

immense capacity for dispersal: shore crabs can be transported over vast distances by tides and currents during their planktonic larval stage, which can last for up to 90 days (Crothers, 1967; Rainbow et al., 1999). Organisms that exhibit this type of dispersal pattern typically lack clear patterns of population divergence due to low levels of genetic differentiation and limited inter-population variability (Hilbish, 1996).

It is therefore surprising that a number of recent studies have detected extensive phenotypic variability in shore crabs within relatively restricted geographical areas. For example, specimens collected from locations around the coast of the UK have been found to differ in terms of their morphology (Bentley et al., 2002; Brian, 2005; Lye et al., 2005) and camouflage pigmentation (Todd et al., 2005). Given that population divergence is prevented by gene flow, these patterns of phenotypic variability are likely to reflect differences between the local environmental conditions, resulting in within-generation selection pressures and/or phenotypic plasticity.

The aim of this study is to assess patterns of morphological and genetic variability in UK shore crab populations in order to investigate the source of this inter-population variability. The decoupling of these parameters will help to establish the extent to which environmental factors influence the expression of phenotypic variability in C. maenas, as well as improving our understanding of the ecology and biogeography of this species. These fundamental issues are of current interest given that the shore crab is a highly successful global invader, having colonised Australia, Tasmania, South Africa, Japan and both coasts of North America (in Roman and Palumbi, 2004). It poses a significant risk to the intertidal ecosystem in these areas, causing changes in the abundance, size structure and defence response of native species (Yamada, 2001).

The expression of inter-population variability in this species is also of interest from the perspective of biomonitoring: the shore crab has recently been championed as a sentinel for assessing the effects of chemical contamination (e.g. Galloway et al., 2004). However, in order to detect the effects of anthropogenic factors, it is important to consider the potential for natural variation in the biomarker response in order to establish the limits of normality (Brian, 2005; Sumpter and Johnson, 2005).

MATERIALS AND METHODS

Adult green shore crabs (classified as those with a carapace width of >30mm) were collected by hand from the inter-tidal zone at eight sites around the coast of the UK (Figure 1). Sites on the west coast consisted of the Dee, Mersey and Clyde estuaries, as well as two Scottish sea lochs, located near Appin and Arisaig. East coast sites comprised the Tyne and Tees estuaries and the Firth of Forth. All specimens were collected within a six-week period during the summer of 2001. Individuals that exhibited symptoms of infection by Sacculina carcinii were discarded. The remaining crabs were transported live, back to the laboratory, in cool-boxes for the morphometric and genetic analyses.

The morphological dimensions are illustrated in Brian (2005). These measurements were made using digital callipers (± 0.01 mm). The length and width of the carapace, and the depth of the cephalothorax, were measured at the widest, longest and deepest points, respectively. Chelae depth was measured at the deepest points and the degree of heterochely was calculated as the depth of the right cheliped minus that of left. The morphology of each claw was also determined by dividing the depth of the

cheliped by its width. Individuals that had lost chelae, or that were suspected to have regenerated one or more cheliped (on the basis of their body size ratio), were omitted from the analysis of claw morphology. Periopod and propodus lengths were recorded by averaging these measurements for the fourth and fifth pairs of limbs, respectively. Again, data from individuals with missing or regenerating limbs were excluded. The area of the abdomen was calculated using the image analysis package, Image Tool V3 (UTHSCSA, San Antonio, USA). The lengths of the major pleopods were determined under a dissecting microscope with a graticule ($\pm 0.2\text{mm}$). The influence of body size was removed by using the residuals for each trait plotted against carapace width as adjusted trait values (Debusse et al., 2001). Where necessary, these values were log-transformed to achieve normality, before being entered into a stepwise discriminant function analysis (DFA) using Statistica V7 (Statsoft) to determine Mahalanobis distances between each pair of sites.

The analysis of genetic population structure was conducted using samples of muscle tissue taken from the chelae of 50 crabs from each site: 25 males and 25 females. Allozyme electrophoresis was performed using horizontal starch gels, run for four hours at -4°C . The gel and bridging buffers were prepared according to Wendel and Weeden (1989) and Creasey et al (1997). Staining protocols were obtained from Cheliak and Pitel (1984). Initially, 14 enzyme systems were trailed using six different buffer systems. This yielded six loci that could be consistently resolved (Table 1). Each zymogram was scored by two independent observers and the alleles were scored according to their rate of migration. There were no differences between the allele frequencies of male and female crabs, which meant that the results from both sexes were pooled. The data were analysed with an online version of the population

genetics analysis package, GENEPOP (Curtin University of Technology, Australia). This was used to determine the genetic structure and heterozygosity of crabs from each population, in addition to estimating the degree of genetic variation among populations. The latter was based on Wright's F-statistics (Weir and Cockerham, 1984), which were used to determine the degree of genetic differentiation between each pair of sites.

Cluster analysis was performed to assess the patterns of morphological and genetic variability among sites. These were plotted in the form of dendrograms, which were constructed according to the unweighted pairgroup method of analysis (UPGMA) in Statistica V7. The relationship between the degree of morphological differentiation (Mahalanobis distance) and the genetic distance (F_{st}) between crabs at each pair of sites was analysed by calculating Pearson's product moment correlation coefficient in Minitab V13.1 (Minitab Inc.). The data was normalised prior to analysis and the correlation was performed independently for male and female crabs to account for differences in their morphology.

RESULTS

The DFA and cluster analysis reveal that there is extensive inter-population variability in the morphology of male and female shore crabs (Figure 2). Differences are more pronounced in males (evident from the difference in the scale on each dendrogram), although the patterns exhibited by each sex are similar. Inter-population differences in the mean values for each trait are presented in Brian (2005). Male crabs from Arisaig, Appin, and the Dee and Clyde estuaries are most alike in terms of their

overall morphology, with males from the Tyne and Tees exhibiting the greatest difference from this group (Figure 2a). Distances between the morphology of female crabs reveal three main clusters: females from the Dee and Clyde are most similar and form a cluster with those from the Mersey. This cluster then joins with another, comprising of females from Arisaig, Appin and the Forth. Tyne and Tees females were most distinct in terms of their morphology (Figure 2b). DFA revealed eight characters that are informative in differentiating between male crabs from each site ($F_{56,1760} = 4.02$, $p < 0.01$). Six of these are significant functions (abdominal area, cephalothorax depth, periopod length, right chelae depth, right chelae morphology and heterochelae). DFA was moderately successful at reclassifying males according to their site of origin: 33.1% of individuals were assigned correctly, compared to the 12.5% that would have been assigned to the correct site by chance. Eight functions are also useful when discriminating among female crabs ($F_{56,1572} = 3.22$, $p < 0.01$), although only three of these are significant (abdominal area, cephalothorax depth and periopod length). 37.3% of females were correctly assigned to site on the basis of their morphological variability.

The analysis of genetic variability reveals that four out of the six loci studied are polymorphic. The PGM and PGI loci exhibit the highest variability: the overall frequency of genotypes, other than the most common, is approximately 25% and 5%, respectively. Low levels of genetic variability (~1%) are found at the AAT and MDH loci. The distribution of genetic variability does not differ between the populations at each site and the observed heterozygosities (Arisaig=0.14; Appin=0.11; Clyde=0.11; Forth=0.10; Tyne=0.09; Tees=0.11; Mersey=0.09; Dee=0.12) are consistent with those expected on the basis of random mating (in line with the Hardy Weinberg

equilibrium). There is no evidence of population sub-division: pairwise F_{st} values, calculated using the data from the four variable loci, are in the order of 0.01. These results indicate that there is very little genetic differentiation among the sub-populations in relation to the meta-population as a whole (Wright, 1943). Despite the low levels of genetic variation, the analysis of patterns of genetic similarity (Figure 3) revealed that the crabs at the Mersey and Dee are most similar, followed by those at Appin and the Clyde. Crabs from Appin and the Clyde form a more distant cluster with crabs from Arisaig. The populations at the Tyne, Forth and Tees are most genetically dissimilar to the crabs in these two groups, as well as to each other. It is interesting to note that the crab population at the Tyne is genetically more similar to those on the west coast than they are to the populations at the Forth and Tees.

The cluster analysis revealed that there were some similarities between the patterns of morphological and genetic variation. For example, Figures 2 and 3 show that crabs from Arisaig, Appin and the Clyde are closely related in terms of their morphology and genetic population structure, whereas crabs from the Tyne and Tees are more distinct in both respects. However, there are notable exceptions. For example, male and female crabs from the Firth of Forth (on the east coast of Scotland) appear more similar to those on the west coast on the basis of their morphology than in terms of their genetic variability. Nevertheless, a moderate correlation between the pairwise differences in morphology and genetic variability was detected in both males ($r=0.47$, $p<0.05$) and females ($r=0.43$, $p<0.05$). Figure 3 indicates that the degree of genetic differentiation may be related to the geographical distance between each pair of sites: crabs from the northwest and southwest form clusters that are less similar to those on the east coast. There is also evidence of a latitudinal cline in the genetic similarity of

crabs on the west coast of the UK. This was investigated by correlating the degree of genetic similarity (F_{st}) with the latitudinal distance between each pair of sites, which revealed that a close association between these factors ($r=0.86$, $p<0.01$).

DISCUSSION

The results of this study provide further evidence that UK shore crab populations exhibit extensive morphological variability, which is consistent with the findings of Bentley et al. (2002), Brian (2005) and Lye et al. (2005). There is evidence that patterns of morphological similarity are, to some extent, related to genetic population structure, although the relationship is not clear. The genetic analyses reveal trends that are typical of decapods i.e. low levels of variability across the shore crab meta-population as a whole, but large variations in the degree of heterozygosity expressed from locus to locus (Tracey et al., 1975). The PGM locus exhibits the greatest variability, with heterozygosities of between 0.33 and 0.5 at each site. The majority of loci, however, have heterozygosities of 0.05 or less, and a high proportion of loci are fixed at all sites. These patterns are consistent with allozyme data describing the population genetics of this species on a larger spatial scale (Bulnheim and Bahns, 1996). There is no evidence of genetic divergence within the crab population at each site, which indicates that there is a high degree of genetic mixing and similar selection pressures. The distribution of genetic variability does, however, reveal evidence of a latitudinal cline, which is likely to reflect larval drift (the movement of adult crabs is more limited). This cline is consistent with ocean circulation patterns, which result in the movement of water in a northerly direction along the west coast of the UK.

The correlation between the pairwise differences in the degree of morphological and genetic similarity of crabs from each site suggests that the phenotypic characteristics of this species are related to patterns of genetic variability (although this relationship is not necessarily causal). The relationship is not strong: the correlation revealed that only around 22% and 18% of the morphological variability exhibited by males and females, respectively, could be linked to the patterns of genetic variability. This suggests that these two parameters are relatively independent and, hence, that the expression of phenotype is largely determined by the environmental conditions. The environment may affect the phenotypic characteristics of a population by selecting for, or against, particular genotypes (Hilbish, 1996). Alternatively, phenotypic variations may arise as a plastic response to the local environmental conditions. Phenotypic plasticity, which is the environment-dependent expression of a genotype, is an evolutionary strategy that enables organisms to adjust their morphology and physiology to match the local conditions within their own lifespan (Bradshaw, 1965; Schlichting, 1986). Although plasticity is usually associated with sessile organisms such as plants (Bradshaw, 1965) and corals (Todd, et al. 2004) that cannot escape their environment once they have become established, reports of plastic responses in motile organisms are not unknown (e.g. Imre et al. 2001; Moore et al., 2004).

The PCA revealed that both male and female shore crabs exhibited the greatest inter-population variability in abdominal area, cephalothorax depth and periopod length. This suggests that variations in the morphology of these characteristics are associated with the differential ability of shore crabs to compete or reproduce in the environment at each site. For example, variations in periopod length may have arisen in response to differences in the local substrate conditions. It is also possible to discriminate

between male crabs from each site on the basis of the size and shape of the right (dominant) chelae. Variations in the morphology of this structure have previously been linked to diet and prey resistance (Smith and Palmer, 1994; Smith, 2004). Differences in chelal morphology are less pronounced in female crabs, which indicates that the patterns observed in this study may be sex linked. The male crab uses the dominant chela as a sexual ornament and as a weapon, in addition to a feeding structure (Mariappan et al., 2000). Variations in the morphology of this trait may be associated with population density, as this is likely to influence the rate and intensity of intra-specific interactions. MacFarlane et al. (2000) reported variations in chelal pigmentation in the semaphore crab, Heloecius cordiformis, which varied with environmental quality. This was attributed to an indirect relationship with population density and levels of inter and intra-specific competition. The chelal morphology of the European lobster, Homarus gammarus, has also been found to vary with population density (Debuse et al., 2001).

Although it is possible to suggest how variations in shore crab morphology could be adaptive in different environments, it is difficult to tease apart the possible causal explanations due to the complexity of the relationship between phenotype and the local physical and synecological conditions. The genetic similarities among crab populations indicate that there is little local adaptation (long term genetic change). Nevertheless, in the period of development from first juvenile instar to adulthood, crabs may encounter site-specific biotic and abiotic selection pressures that result in the differential survival of individuals on the basis of their morphology (within-generation selection). For example, both predation pressure and physical environmental factors could influence mean trait values by removing maladaptive

phenotypes (De Witt et al., 1998). Alternatively, or concurrently, the morphology of this species may respond plastically to site-specific selection pressures, causing changes in the expression of particular morphological traits. For example, manipulative diet experiments have revealed plasticity in the claw size of decapods, including Cancer productus and Callinectes sapidus (Smith and Palmer, 1994; Davis et al., 2005), and evidence from the USA indicates that C. maenas has responded, in a plastic manner, to geographic differences in the prey armor of Littorina obtusata, which is a key prey species (Smith, 2004).

Although larval shore crabs are strong swimmers (Hedvall et al., 1998), they have limited control over where they settle. Given their generalist genotype, combined with the environmental heterogeneity associated with their native habitat, the ability to respond to challenges in this plastic manner is likely to be a highly advantageous strategy. This has probably contributed to the invasive capability of the shore crab (Roman and Palumbi, 2004) and indicates that the accidental introduction of its larvae to alien environments will continue to pose a threat to the integrity of the coastal environment on a global scale. The apparent plasticity exhibited by this species also has implications in terms of biomonitoring. Low genetic variability indicates that patterns of phenotypic variability among shore crab populations are likely to reflect differences between local environments. However, there is a risk that the effects of exposure to anthropogenic stressors on phenotype may be masked by natural inter-population variability (Brian, 2005). Hence, the results of this study demonstrate that the expression of inter-population variability in the morphology of C. maenas has both environmental and genetic components that need to be accounted for in future biomonitoring, taxonomic and population-level research.

ACKNOWLEDGEMENTS

This work was funded by Napier University. The authors would like to thank Jennifer Batty and Trevor Blackall for assisting with the collection of crabs and Richard Ennos for providing the facilities required to perform the genetic analyses.

Table 1. Enzyme and buffer systems that provided the best resolution for allozyme analysis using horizontal starch gel electrophoresis.

Enzyme (abbreviation)	E.C. Number	Buffer System	No. Loci	Alleles per locus
Phosphoglucomutase (PGM)	2.7.5.1	Morpholine/Citrate pH 6.1	1	2
6 Phosphogluconic dehydrogenase (6PGD)	1.1.1.44	Morpholine/Citrate pH 6.1	1	1
Malate dehydrogenase (MDH)	1.1.1.37	Morpholine/Citrate pH 6.1	2	2
Aspartate aminotransferase (AAT)	2.6.1.1)	Lithium Borate pH8.2	1	2
Phosphoglucose isomerase (PGI)	5.3.1.9	Tris/citrate pH 8.3	1	3
General Protein (GP)	-	Tris/citrate pH8.3	1	1

Buffer systems described in Wendel and Weeden (1989) and Creasey et al. (1997).

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FIGURE LEGENDS

Figure 1. Locations of sampling sites around the UK. Arisaig: 56° 53' N, 5° 53' W; Appin: 56° 33' N, 5° 24' W; Forth: 56° 00' N, 2° 32' W; Dee: 53° 21' N, 3° 10' W; Clyde: 55° 58' N, 4° 41' W; Mersey: 53° 19' N, 2° 51' W; Tyne: 55° 00' N, 1° 25' W; Tees: 54° 35' N, 1° 13' W.

Figure 2. Dendrograms illustrating the patterns of morphological similarity among male (a) and female (b) shore crabs from eight sites around the coast of the UK. Clustering is based on UPGMA.

Figure 3. Dendrogram illustrating the patterns of genetic similarity among eight UK shore crab populations. Clustering is based on UPGMA.

Figure 1

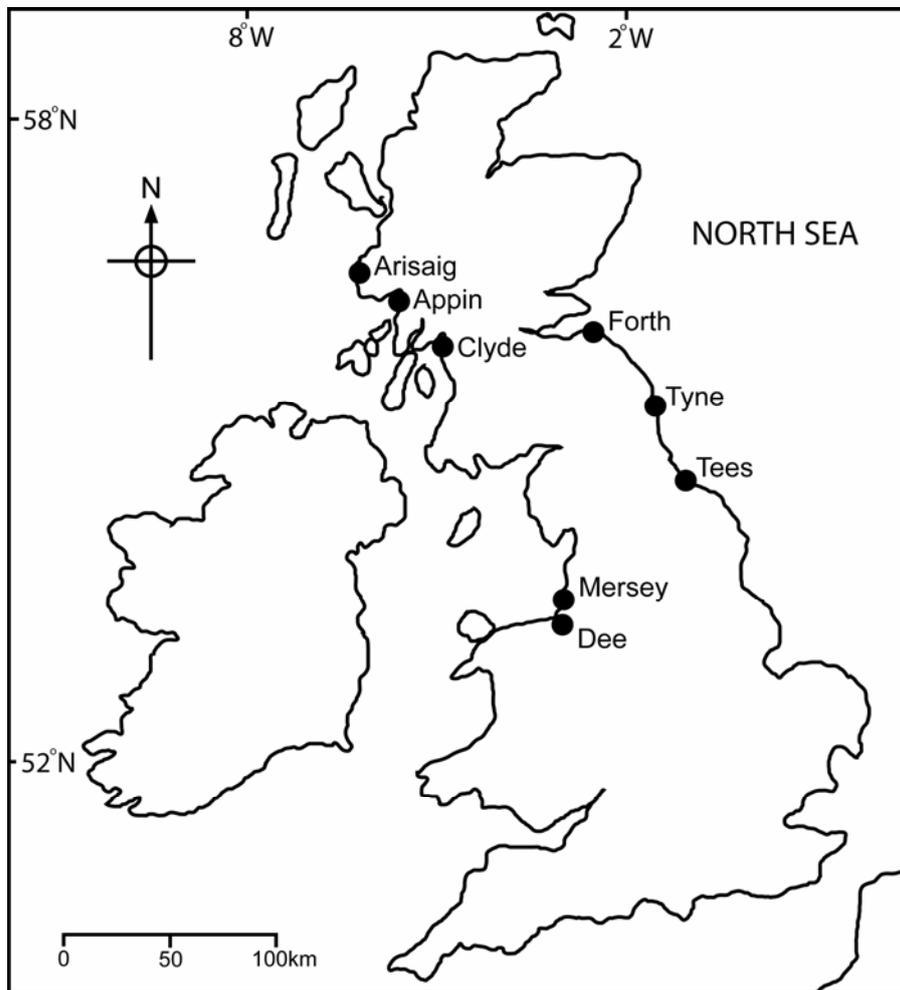


Figure 2

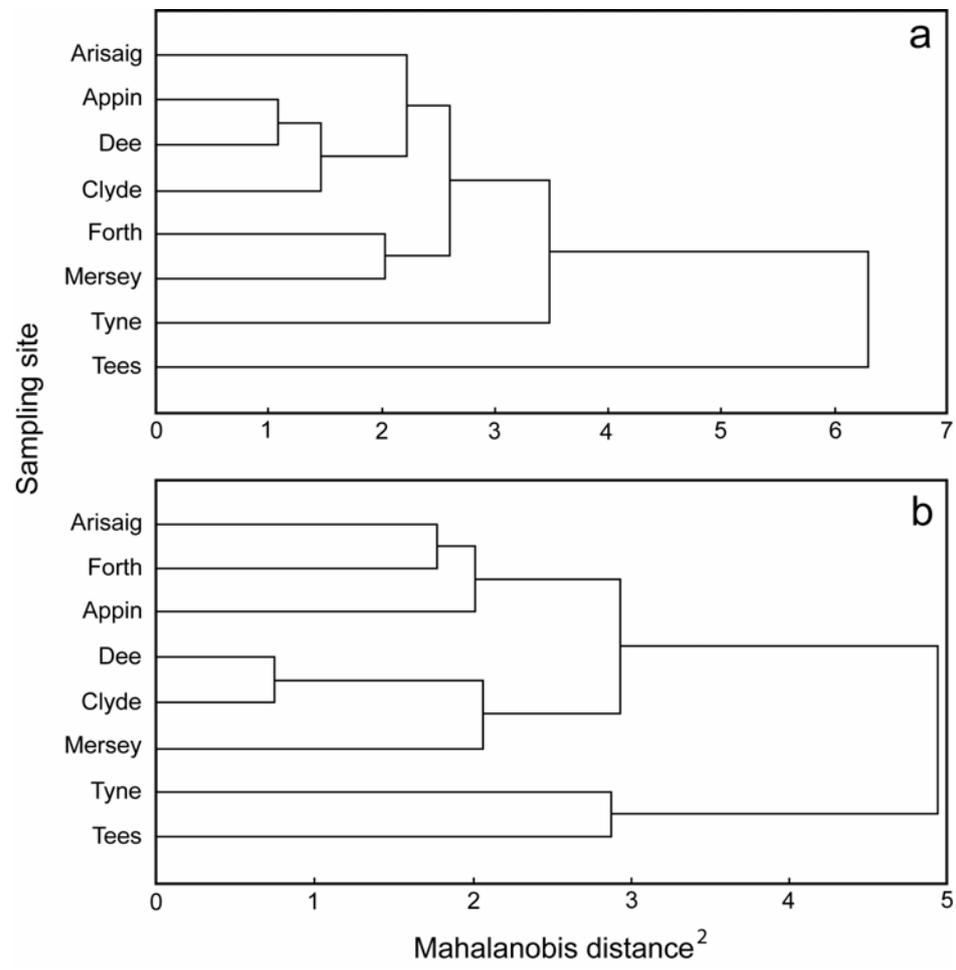


Figure 3

