ORIGINAL ARTICLE

Using molecular and quantitative variation for assessing genetic impacts on *Nucella lapillus* populations after local extinction and recolonization

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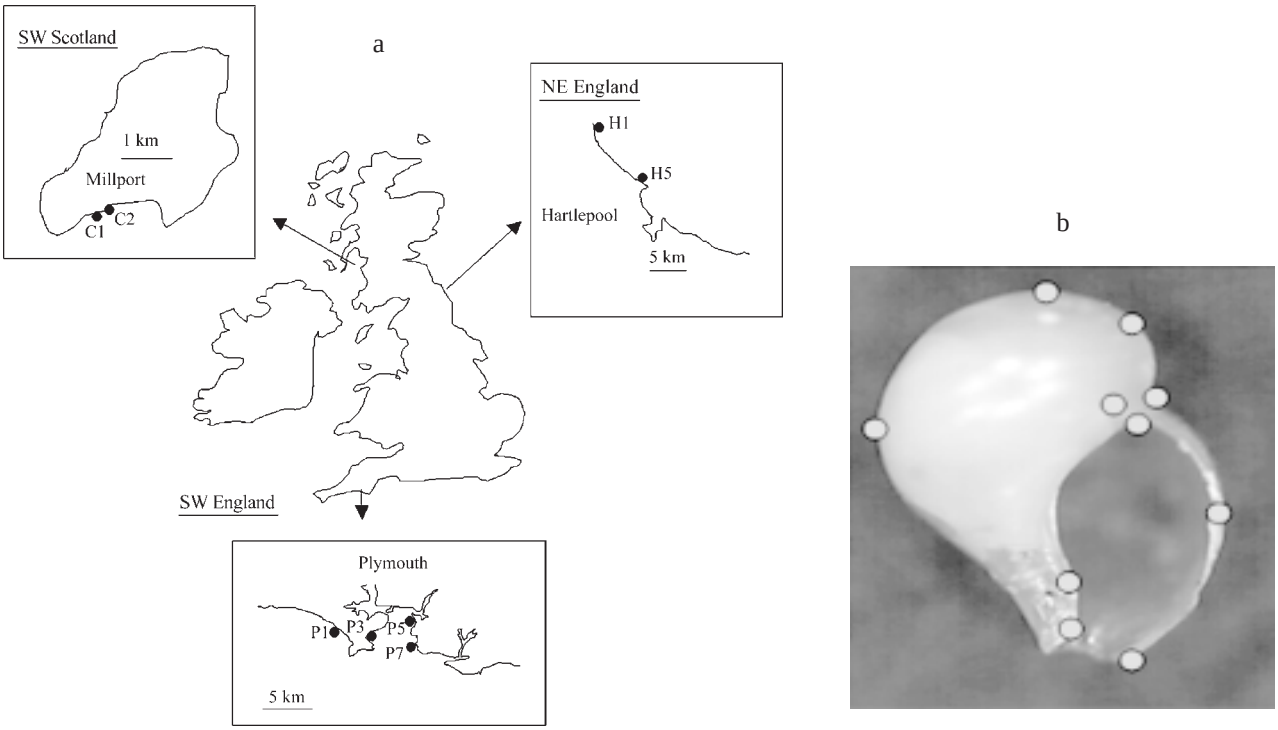

Figure 1 (a) Location of the sampling sites; (b) landmarks used for studying shell form in embryos within each capsule.

populations, probably due to the homogenizing effects of migration.

MATERIALS AND METHODS

Dogwhelks were collected from three localities across the British Isles (Fig. 1a). At each locality we collected individuals from sites where the demographic history of *N. lapillus* is well-documented (Hawkins *et al*. 2002), including sites where extinction/recolonization events occurred and sites that have shown continuous population during the whole survey period, since 1986. Twenty-four adult individuals were collected for genetic analysis from eight sites during May-June 2002 (except one site collected in May 2003), and stored in ethanol until DNA extraction.

Molecular variability was estimated by genotyping six

Table 1 Continuous and recolonized populations studied in three localities, with sample sizes for both molecular and the quantitative analyses

Area	Locality	Treatment	Specimens analysed	He	Na	Number of capsule (shelled embryos)	VA	Coefficient of additive variation
	P1	Continuous	24	0.62	6	18 (90)	0.0700	0.1450
	P3	Recolonized	24	0.48	5.00	2 (10)	0.0230	0.0967
Plymouth	P5	Recolonized	24	0.58	4.83	20 (100)	0.0260	0.0958
	P7	Continuous	24	0.57	5.50	17 (85)	0.0120	0.0752
Isle of	C1	Continuous	24	0.56	6.33	5 (12)	0.1580	0.2464
Cumbrae	C2	Recolonized	24	0.58	5.83	14 (61)	0.0100	0.0677
Hartlepool	H1	Continuous	24	0.50	5.67	4 (20)	0.0420	0.1330
Area	H5	Recolonized	24	0.48	5.50	25 (123)	0.0370	0.1286

He, heterozygosity; Na, no. alleles; VA, mean additive variation for the four principal components.

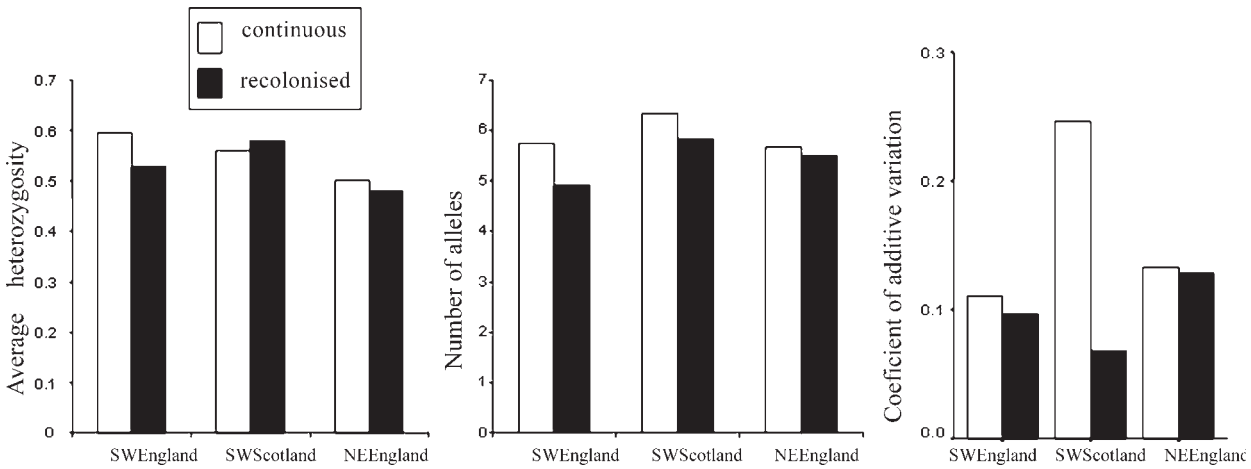

Figure 2 Average heterozygosity, number of alleles and coefficient of additive variation for continuous and recolonized sites from the three studied localities.

microsatellite loci. DNA was extracted from foot tissue and amplified for the following loci: Nlw2, Nlw3, Nlw8, Nlw21, Nlw25, Nlw27, described in Kawai *et al*. (2001). The amplified fragments were sized on acrylamide gels on an ABI377 automated sequencer (Applied BioSystems, California). Average heterozygosity, using the expected heterozygosity $He = [n/(n-1)] (1-\Sigma x_i^2)$, and mean number of alleles across loci were calculated for each population.

Nucella lapillus is an oviparous gastropod with direct development. A group of capsules (with 2-20 shelled embryos per capsule) may be deposited by the female during the breeding season. Quantitative genetic estimates of variation on shell morphology can be obtained by partitioning the population shell variance in two components: between (σ_b^2) and within (σ_w^2) family components (Carballo *et al*. 2001). Therefore, assuming full sibship within families, the heritability (proportion of additive variation) is double the ratio between the aforementioned components ($\sigma_b^2/[\sigma_b^2 + \sigma_w^2]$). Using a digital video on a Leica stereo microscope, we recorded the shell image of approximately five shelled embryos per capsule in a variable number of capsules per population (Table 1). For each shelled embryo we measured distances between all the unique pairs of 10 standard landmarks (Fig. 1b). These 45 distances were reduced by principal components analysis to four independent variables (PC1–PC4) that represented the majority of variation in shell form (>90% of total variation). Heritability and additive variance estimates could be biased by dominance and the common environmental variance components. However, each capsule was maintained in the laboratory for a week during its maturation, in an attempt to homogenize the environmental effects contributing to the differences between families. This analysis assumes that embryos within capsules are full sibs, which, if incorrect, may also bias estimates per sample. However, neither of the aforementioned two sources of bias should differentially affect recolonized and continuous populations, and so the methodology can be used for approximate comparison of levels of quantitative genetic variation between the two classes of population.

RESULTS

Relatively few sampled capsules contained mature shelled embryos and so the sample size of families was low for most sites (Table 1). Nevertheless an informative trend was observed, as follows. The mean heritability for the four main principal components was 0.509 (SE: ± 0.114) across all the samples. There was a positive and nearly significant correlation between the additive variance and the mean number of alleles across microsatellite loci ($r = 0.689$, d.f. = 7, $P = 0.059$) but no other significant correlation. The comparison between continuous and recolonized populations for heterozygosity, number of alleles and coefficient of additive variation (corrected for differences in means among samples) are shown in Figure 2. The trend was broadly similar for the three estimators of genetic diversity: recolonized populations presented slightly lower genetic variability than continuous populations in the three studied areas, although in SW Scotland, mean heterozygosity was slightly higher in the recolonized population.

DISCUSSION

Extreme bottlenecks are theoretically predicted to decrease the genetic diversity of the affected population, although it is known that the expected decline in heterozygosity can be compensated for by the homogenizing effects of migration (Hawkins *et al*. 2002). Our data show a slight decrease in both molecular and quantitative variation, although the small number of observations does not allow powerful statistical analyses. The lack of significance of the results obtained in quantitative variation could be partially caused by the few data available (Table 1), but both molecular and quantitative variation suggest that the overall reduction in genetic diversity may be small. The simplest explanation for this absence of clear reduction in genetic variation in populations affected by a bottleneck is that levels of genetic diversity have been recovered a posteriori due to relatively high levels of migration. *Nucella lapillus*, lacking a planktonic larval phase, is expected to show limited dispersal. However, the fast recovery of genetic diversity after extinction/recolonization events observed here indicates that the dispersal ability of *Nucella* might be higher than generally assumed. A detailed comparative analysis of the genetic structure in marine organisms revealed that dogwhelk populations show less genetic structure than most direct developing species for which genetic data are available (Colson & Hughes 2004). These observations lend support to a model of relatively high dispersal ability of *Nucella*, considering its life history characteristics.

Perhaps the most interesting contribution of this study is to show the potential use of quantitative variation for assessing genetic effects of anthropogenic activities. This is a preliminary study based on a limited number of comparisons (only six populations), but the correlation between additive variation and mean number of alleles suggests that both could be similarly impacted upon during the demographic history of these populations. Furthermore, the estimated heritability and additive variances fall within the range obtained in related gastropods (Carballo *et al*. 2001). Quantitative estimates, when possible, can be a cheap alternative producing complementary information to the molecular markers.

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