

Modelling the genetics and demography of step cline formation: gastropod populations preyed on by experimentally introduced crabs

E. G. BOULDING, T. HAY, M. HOLST¹, S. KAMEL², D. PAKES³ & A. D. TIE⁴

Department of Integrative Biology, University of Guelph, Guelph, ON, Canada

Keywords:

demography;
experimental evolution;
field experiment;
fitness function;
Hemigrapsus nudus;
invading species;
Littorina subrotundata;
population structure;
quantitative genetics;
theory.

Abstract

Whether a prey population goes extinct or adapts in response to an invading predator may depend on the number of contiguous populations that experience increased predation. We created invaded snail populations by building shelters for predatory shore crabs on a rocky intertidal bench. The crabs preyed selectively on thin-shelled snails tethered next to the shelters but did not prey on those more than 2 m away. This caused strong directional selection for increased shell thickness in populations close to the shelters but did not change selection in those farther away. The field experiment was used to parameterize a new individual-based quantitative genetic model that included demography. In the model a detectable step cline in shell thickness evolved rapidly even though the region of increased predation was shorter than Slatkin's *characteristic length*. The cline's step size in the model was similar to that measured in the field 10 years after the experiment began.

Introduction

Human technology has greatly increased the rate at which nonindigenous predator species are introduced into aquatic ecosystems (Enserink, 1999). Establishment of a predator species in a new region can substantially change selection on indigenous prey populations and cause them to rapidly decline towards extinction (Zaret & Paine, 1973; Atkinson, 1977; Clarke *et al.*, 1984). However, theoretical studies suggest that if a declining prey population can rapidly evolve defences against the predator then it will increase back up to the carrying

capacity and survive the invasion. Indeed the dynamics of a prey population after a single-step (or discrete) change in its optimal phenotype have been modelled as a race between demographic and evolutionary pressures (Gomulkiewicz & Holt, 1995; Boulding & Hay, 2001). A population can also adapt to continuous change in the optimal phenotype if the rate of change is slow and if demographic and genetic parameters are favourable (Pease *et al.*, 1989; Lynch & Lande, 1993). These theoretical studies are supported by empirical evidence that rapid evolution of complex or quantitative traits has occurred in response to changes in natural selection from predators. For example, guppies transplanted to pools above waterfalls where predation was low showed changes in life-history traits after seven to 18 generations (Reznick *et al.*, 1997), and marine littorinid snail populations developed a thicker, more predator-resistant shell within 17 generations after being invaded by a predatory shore crab (Seeley, 1986).

Rapid evolution of a quantitative trait in response to an invading predator can be reduced or entirely prevented by gene flow from predator-free populations (Boulding & Hay, 2001). Similarly, gene flow can prevent populations on the periphery of a species' range from adapting to their local environment (García-Ramos & Kirkpatrick,

Correspondence: E. G. Boulding, Department of Integrative Biology, University of Guelph, Guelph, ON, Canada N1G 2W1.
Tel.: +1 519 824 4120; ext: 54961; fax: +1 519 767 1656;
e-mail: boulding@uoguelph.ca

Present addresses:

¹LGL Ltd, Environmental Research Associates, 9768 Second St, Sidney, BC, Canada V8L 3Y8.

²Department of Zoology, 25 Harbord St, University of Toronto, Toronto, ON, Canada M5S 3G5.

³The Koret School of Veterinary Medicine, POB 12, Rehovot 76100, Israel.

⁴Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, ON, Canada N1G 2W1.

1997), which can cause them to become demographic sinks (Kirkpatrick & Barton, 1997). This has been referred to as 'migrational meltdown' (Ronce & Kirkpatrick, 2001). High migration can prevent a quantitative trait in a population that inhabits a stepped environmental gradient from forming a cline (Slatkin, 1978). A cline will form only if the patch of homogeneous habitat exceeds the 'characteristic length' which Slatkin (1978) defined as the smallest step change in the environment to which the local population can adapt. Recent quantitative genetic models of clines now include demography but do not consider adaptation to a nonlinear environmental gradient (Kirkpatrick & Barton, 1997; Alleaume-Benharira *et al.*, 2006).

The inclusion of demography in models of clines makes them more difficult to solve, parameterize and validate. Validation of theory by comparison to field data is rare for quantitative genetic models (Hendry *et al.*, 2001; García-Ramos & Rodríguez, 2002; Pérez-Figueroa *et al.*, 2005), and almost nonexistent for models that include demography (Tufto, 2001). Most quantitative models with demography are unable to determine whether the regions of parameter space with interesting model dynamics are biologically relevant because, as pointed out by Tufto (2001), suitable parameter estimates do not exist. Consequently, it is important to find out when including demography in models is necessary. This can be accomplished by using experimental systems to parameterize and validate quantitative genetic models. Here, we compare the predictions of two quantitative genetic models to the experimental results from a system comprising the predatory shore crab, *Hemigrapsus nudus*, and the herbivorous marine snail, *Littorina subrotundata*.

Invasions of predatory crabs into indigenous molluscan prey populations are often patchy, creating pockets of invaded populations surrounded by predator-free populations. The introduced green crab, *Carcinus maenas*, has a very patchy distribution on the Pacific coast of North America (Cohen *et al.*, 1995; Grosholz & Ruiz, 1995). However, where this crab is abundant, it selects for thicker shelled gastropods (Seeley, 1986). Evolution of increased shell thickness will increase the resistance of gastropod prey not only to this crab species but also to many other species of shell-crushing predators (Vermeij, 1977, 1987; Boulding *et al.*, 1999).

Marine intertidal gastropod species in the genus *Littorina* are ideal for testing models of local adaptation because they are abundant, slow moving and their shells are easily marked. Species that lack a free-swimming larval stage are particularly useful (reviewed in Reid, 1996) because their population dynamics and population genetics respond to local abiotic and biotic environmental conditions (Boulding & Harper, 1998; Sokolova & Boulding, 2004). For example, adjacent populations of *L. saxatilis* often show genetic differences in shell size and thickness in response to vertical and horizontal microgeographical gradients in predation (Johannesson *et al.*,

1993; Rolán-Alvarez *et al.*, 1997, 2004; Wilding *et al.*, 2001).

In this paper we use a field experiment to parameterize and validate a new 'finite locus' quantitative genetic model of the demography and genetics of a linear series of snail populations. In the model, two to six adjoining central populations experience an invasion by a new predatory crab that selects for increased shell thickness. This simulated invasion can result in the evolution of a double-sided step cline for shell thickness because there is selection for the predator-resistant morphology in the central 'core' invaded populations and selection for the original morphology in the peripheral populations. In the field experiment shelters were constructed for predatory shore crabs near the centre of previously predator-free sites to create selection for predator resistance. We used this experimental gradient to estimate parameters for our finite locus model, as well as for Slatkin's (1978) analytical model. We then used the parameterized models to predict the evolutionary change in shell thickness in the snail populations near the crab shelters. Finally, we validated the models by comparing the shell thickness predicted by the parameterized models with the observed evolutionary response of snail populations near the crab shelters 10 years after the beginning of the field experiment (Dalziel & Boulding, 2005).

Materials and methods

Field experiment

Study sites

We created a stepped along-shore gradient in predation for snail populations living in the barnacle (*Balanus glandula*) zone near Bamfield Marine Sciences Centre (48°50'N, 125°08'W) on the west coast of Canada. We chose to manipulate predatory crab density at Nudibranch Point and at Prasiola Point because these sites: (1) are wave-exposed which eliminates most marine predators on littorinid gastropods (Boulding *et al.*, 1999); (2) have large populations of the intertidal snail *L. subrotundata* (Carpenter 1864); and (3) are accessible by boat in winter as well as in summer. We increased the density of predatory crabs by building concrete shelters in the centre of our two sites in September 1993 (Supplementary Material: Appendix S1, Fig. S1, Table S1). We then collected purple shore crabs, *H. nudus* (Dana, 1851), between 15- and 25-mm carapace width from nearby wave-sheltered sites and introduced one crab into each shelter opening. At each site we marked vertical 1-m transects by placing stainless steel screws into holes drilled into the rock at different distances from the shelters. Each transect was composed of five permanent 10 cm × 10 cm quadrats. We used the quadrats to monitor the demography and genetics of the surrounding natural snail populations as a function of distance from the shelters.

Study animals

We chose to focus on *L. subrotundata* because its small adult size and thin shell make it vulnerable to even small shell-crushing predators. Consequently, it is only abundant at wave-exposed sites and other locations where shore crabs are rare (Boulding & Van Alstyne, 1993; Kyle & Boulding, 1998). An ecologically similar but larger and thicker shelled species, *L. sitkana*, Philippi, 1846, was rare at our sites but is abundant on wave-protected shores where shore crabs are common (Boulding & Van Alstyne, 1993; Boulding *et al.*, 1999). The former two species of *Littorina* have direct development from attached egg masses. However, two other littorinid species that were abundant, *L. plena*, Gould, 1849, and *L. scutulata*, Gould, 1849, as well as the predatory crab, *H. nudus*, have a feeding, or planktotrophic, larval stage that swims freely in the plankton for at least 5 weeks (Reid, 1996; Strathmann, 1997; Hohenlohe, 2002). Planktotrophic development has been shown to result in extensive dispersal in these or closely related marine species (Kyle & Boulding, 2000; Cassone & Boulding, 2006) which makes local adaptation less likely. Consequently, we did not include coevolution of the predatory crab or of its alternative prey species in our model.

Analytical model of cline

Slatkin (1978) described an analytical model of a cline in a quantitative trait that was subject to a small step change in the optimal phenotype such as we created in our field experiment. He found that for weak selection and symmetric dispersal, adaptation to local conditions took place when the change in the optimum occurred over distances greater than l_c , the characteristic length. Substituting equation 11 in Slatkin (1978) into his equation 23 but using the notation described here, we get the simple but elegant expression:

$$l_c = \frac{\sigma}{\sqrt{\frac{h^2 V_z}{V_z + \omega^2}}} \quad (1)$$

where σ is the SD of life-time dispersal displacements, h^2 is the heritability, V_z is the phenotypic variance and ω is the SD of the Gaussian fitness function, all of which he assumed were constant throughout the cline. However, Slatkin's (1978) model did not consider strong selection or demography and consequently could not compute the risk of extinction of the invaded populations. Therefore, in addition to his model, we also used a finite locus model employing computer simulation of the loci within each individual snail.

Multipopulation finite locus model

The finite locus model also considers a quantitative trait subject to a step change in the optimal phenotype. The model is an extension of a single population model

(Boulding & Hay, 2001) to a one-dimensional series of 19 populations connected by migration and uses the same computer program. For more details of the model see Supplementary Appendix S2. The model assumes that a discrete environmental change, in this case predator invasion, in k -adjacent central populations, results in a quantitative trait being selected towards a new optimum, θ_c . The number of populations in the central 'core' invaded area, k , was 2, 4 or 6.

In each generation of a simulation, the ordering of events was the same: (1) the offspring were created and subjected to viability selection; (2) their parents died; (3) the population size of the surviving offspring was recorded; and (4) migration occurred. The complete cycle was repeated for each generation until the central core populations had gone permanently extinct or had recovered back up to the carrying capacity.

The growth rate of each population in the model was density independent up to the ceiling of N_{\max} . This was possible because offspring were produced one at a time by: (1) choosing two parents at random; (2) generating a gamete from each diploid parent by choosing one allele at each of its 96 loci; and (3) combining the two gametes locus by locus to create the offspring's genome (Boulding, 1990). The average fecundity (f) of each adult was 66 (Table 1). Therefore, the maximum number of offspring that could be produced was the minimum of N_{\max} and $N_t \times f$, where N_t was the current population size of the parent generation.

Fitness was determined by viability (or hard) selection in that the probability, $W_{i,x}$ of juvenile i surviving to become an adult in population x was determined by the Gaussian fitness function:

$$W_{i,x} = \exp \left[-\frac{(z_{i,x} - \theta_x)^2}{2\omega_x^2} \right] \quad (2)$$

where $z_{i,x}$ is the value of the trait for individual i in population x , θ_x is the optimal value of z at distance x , and where ω_x is the width (or SD) of the Gaussian fitness function at distance x . The phenotype $z_{i,x}$ was made up of a genetic component (or breeding value), $g_{i,x}$, determined by 96 loci of equal effect, and of a random environmental component, $e_{i,x}$. When $\bar{z} = \theta_x$ then ω_x^2 was inversely proportional to the strength of stabilizing selection towards the optimum. When $\bar{z} < \theta_x$ then the population experienced a mixture of positive directional and stabilizing selection. Multiple step migration occurred right after viability selection during which $m_j \times N_x$ individuals left the focal population x for the populations on each side that were j populations (or steps) away (Table 1). Previous work has shown that varying the number of loci from eight to 1024 did not substantially change the rate of evolution (Boulding & Hay, 2001). An asset of a finite locus model is that it automatically incorporates genetic drift and linkage disequilibrium; Pérez-Figueroa *et al.* (2005) obtained a better fit to their phenotypic data for

Table 1 Means and standard errors of biologically realistic parameters estimated from field experiment.

Parameter name	Symbol	Parameter mean (standard error)	Reference
Phenotypic standard deviation (PSD) equivalent to 3.2 mg shell weight	V_z	1.0 PSD	Table S3
Fecundity	f	66.1	Appendix S1
Width of fitness function			
Before invasion	ω_b	1.0 (0.26) PSD	Appendix S1
After invasion: medium size class	ω_c	2.60 (0.65) PSD	Fig. 1c; Table S2
After invasion: large size class	ω_c	1.71 (0.059) PSD	Fig. 1d; Table S2
Shift in optimal phenotype:			$\Delta\theta = \theta_c - \theta_i$
Medium size class	$\Delta\theta$	2.44 (0.60) PSD	Table S3
Large size class	$\Delta\theta$	3.65 (1.06) PSD	Table S3
Maximum population density	N_{\max}	1000 m ⁻¹	Boulding & Harper (1998)
Initial subpopulation density	N_i	1000 m ⁻¹	Boulding & Harper (1998)
Narrow sense heritability	h^2	0.3 (0.13)	Boulding & Hay (1993)
Number of the 19 populations in 'core'	k	4/19	Appendix S1
SD of life-time dispersal displacements (direct from tagging*)	σ	5.8 m per generation	Table 2
Migration rate* from population i to a population that is j steps away.	$m_j, j = 1-18$	(0.10, 0.07, 0.05, 0.03, 0.02, 0.01, 0.01, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)	Table 2

* j is the number of metres moved by proportion m_j snails in 6 months as estimated by a negative binomial dispersal kernel fitted to the data from the 3.6 m W transect (Table 2).

Littorina saxatilis inhabiting a steep environmental gradient with a 30-locus finite locus model than with an infinitesimal model.

To initialize the model, the first 20 generations were run with the fitness function at an initial optimum, θ_i , of 0 and SD, ω_b , of 1 for all populations. During generation 21, the fitness function of the k 'core populations' invaded by the crabs was shifted to its new optimum, θ_c , and new SD, ω_c , in a single step whereas the fitness function for the surrounding populations remained at its original values of θ_i and ω_b . Population size was always at the carrying capacity, N_{\max} , for the initial 20 generations. However, after the shift in the optimum occurred it was allowed to decline below N_{\max} . The model is general and can be used to predict evolution of any quantitative trait in response to discrete environmental change within a series of populations. Here it was parameterized with data from our field experiment (Table 1). To investigate how cumulative errors in input parameter estimation might affect our predictions we carried out a parametric bootstrapping exercise (Cheng, 1995) on five of our parameters: m , h^2 , $\Delta\theta$, ω_c and ω_b (Supplementary Appendix S1).

Parameter estimation

Fitness function

The methods used to estimate model parameters from our field experiment are summarized here and expanded on in Supplementary Appendix S1. We could not directly estimate the optimum, θ_i , and the SD, ω_b , of the pre-invasion Gaussian fitness function so for simplicity assumed that θ_i was equal the observed mean and ω_b

was equal to the observed phenotypic standard deviation (PSD) of the distribution of the shell weights of *L. subrotundata* of the medium size class (Supplementary Appendix S1).

We estimated $\Delta\theta$ for the invaded populations as the difference between the mean shell thickness of *L. sitkana*, θ_c , and the mean shell thickness of *L. subrotundata*, θ_i . This assumes the shell thickness of the former thick-shelled species is optimal for an environment with predatory shore crabs and that the latter thin-shelled species is optimal for an environment without crabs. The SD, ω_c , of the post-invasion fitness function was estimated by comparing the survival of the two snail species when tethered at different distances from the crab shelters. We tethered two different size classes of snails (medium, 3.36–3.96 mm shell width and large, 3.96–4.76 mm shell width) (Table S2) corresponding to the evolution of increased shell thickness by *L. subrotundata* equal to that of the medium size class of *L. sitkana* or the large size class of *L. sitkana* respectively. To facilitate comparison with the model as well as between the two species, all estimates of shell weight were transformed to PSD by dividing them by 3.2 mg, the SD of shell weight for medium *L. subrotundata* (Table S3).

Migration rates

We estimated the SD of life-time dispersal displacements, σ , using both direct (mark and recapture) and indirect (microsatellite genotyping) methods. For the direct method we collected 500 *L. subrotundata* from the 3.6 m West (W) transect and 404 from the 2.8 m East (E) transect (Table 2). We then completely marked the shell of each snail with orange and yellow paint respectively.

Table 2 Migration experiments at Nudibranch Point to estimate the SD of life-time dispersal displacements, σ , and the dispersal kernel.

Location	No. recaptured	Median (mean) distance (m)	SD distance (m)	Kurtosis (K)	σ^* (m)	σ^\dagger (m)	Kernel coeff.‡
3.6 m West (August)	177	0.71 (1.03)	0.987	6.788 (2.7)	2.0	2.7	
3.6 m West (January)	43	1.11 (1.73)	1.71	0.606 (3.4)	3.1	5.8	$a = 0.361$ $b = -0.416$
2.8 m East (August)	65	0.40 (0.547)	0.564	2.182 (3.0)	1.1	1.7	
2.8 m East (January)	19	0.56 (0.812)	0.676	-0.452 (3.5)	1.6	2.4	$a = 0.231$ $b = -0.610$

Five hundred *Littorina subrotundata* of 2.5–5.5 mm shell length were marked with orange paint at the 3.6 m W transect and were released on 14 June 1999 and 404 were marked with yellow paint at the 2.8 m E transect and were released on 5–6 July 1999. Only the net displacement parallel to the shore's edge from the original release point is shown. Recapture periods were 9–12 August 1999 and 26–30 December 1999. The constant, K , is a function of the kurtosis using table 12.1 in Wright (1969).

*Method 1 of Wright (1969): $\sigma = 2.8 \times$ median dispersal distance.

†Method 2 of Wright (1969): $\sigma = K \times$ SD, where K is a coefficient calculated from the kurtosis (Table 12.1 in Wright 1969) and SD is the standard deviation of the net distance moved.

‡Dispersal kernel coefficients a and b for a negative binomial distribution fitted to the proportion of the snails recaptured that had travelled a particular number of metres in each of two directions. The snails did not migrate farther than 8 m therefore in the smoothed distribution: $m_9 = m_{10} = \dots = m_{18} = 0$. Snails move in each direction from population i . The smoothed migration vector from the 3.6 m W release point was primarily used in the model because it was based on a higher recapture rate than the migration estimates from the 2.8 m E release point.

§Smoothed vector for 3.6 m W for which the fit to the distribution was $r^2 = 0.912$. Migration rate estimate, m_j , where j is the number of metres or (steps) moved by the proportion m_j snails in 6 months: $m_1 = 0.10$, $m_2 = 0.07$, $m_3 = 0.05$, $m_4 = 0.03$, $m_5 = 0.02$, $m_6 = 0.01$, $m_7 = 0.01$, $m_8 = 0$.

¶Smoothed vector for 2.8 m E for which the fit to the distribution gave $r^2 = 0.957$. Migration estimate, m_j , where j is the number of metres or (steps) moved by the proportion m_j snails in 6 months: $m_1 = 0.063$, $m_2 = 0.034$, $m_3 = 0.019$, $m_4 = 0.010$, $m_5 = 0.005$, $m_6 = 0.003$, $m_7 = 0.002$, $m_8 = 0$.

Once the paint was dry we released the snails within 1 m of where they were collected (Table 2). We searched for surviving snails after 1 month and recorded their net displacement parallel to shore without disturbing them. All surviving snails were recaptured after a total of 6 months had elapsed. We then estimated a dispersal kernel by fitting the proportion of snails travelling a given number of metres after 6 months to a negative binomial distribution. This estimated a multiple stepping-stone, migration vector that could be used directly in the finite locus model (Table 2).

For the indirect estimates of migration, we collected *L. subrotundata* from all seven transects at Nudibranch Point and genotyped each snail for six microsatellite loci (Tie *et al.*, 2000). We tested for isolation by distance using the program GENEPOP, which uses the Mantel test to calculate the correlation between the matrix of $F_{st}/(1 - F_{st})$ for each pair of populations and the matrix of geographical distances between each pair of populations (Raymond & Rousset, 1995). The program also calculates the slope of regression of $F_{st}/(1 - F_{st})$ between each pair of transects against the physical distance between them in metres (Tables S4 & S5). The inverse of the slope or $1/b$, estimates $4N_e\sigma^2$ for a one-dimensional population (Rousset, 1997) therefore $\sigma^- = (4N_e b)^{-1/2}$. We assumed that the snails occupied a one-dimensional population that was 1-m wide which enabled us to estimate N_e per linear metre from N_c , the density per square metre estimated from our quadrat counts. This assumes that $N_e = N_c$. However, the positive F_{is} values averaged over the six

microsatellite loci (Table S5) suggest that inbreeding had occurred at the level of the transect (Robertson & Hill, 1984) possibly because of the physical proximity of half-siblings from the same egg mass. In addition to inbreeding, factors such as fluctuations in population size, unequal sex ratios or variance in family size (Freeman and Herron 2006) could cause N_e to be smaller than N_c which would increase our estimate of σ .

Other parameters

The size of the core area, k , was estimated from the proportion of the study area on which predation of tethered snails had been observed. Fecundity per capita was measured by culturing pairs of *L. subrotundata* in tanks and counting the number of eggs per egg mass. Shell weight has been previously estimated to have a narrow sense heritability of 0.3 in a north-eastern Pacific population of *L. subrotundata* (Boulding & Hay, 1993).

Results

Parameter estimates from field experiment

Field experiment

The field experiment successfully created a stepped environmental gradient in predation. The shore crabs foraged almost exclusively on snails tethered within 2 m of the concrete shelter that they inhabited. Nearly all the mortality of tethered snails that resulted in crushed shells occurred at the 0.5 m W and 1.5 m E transects right next

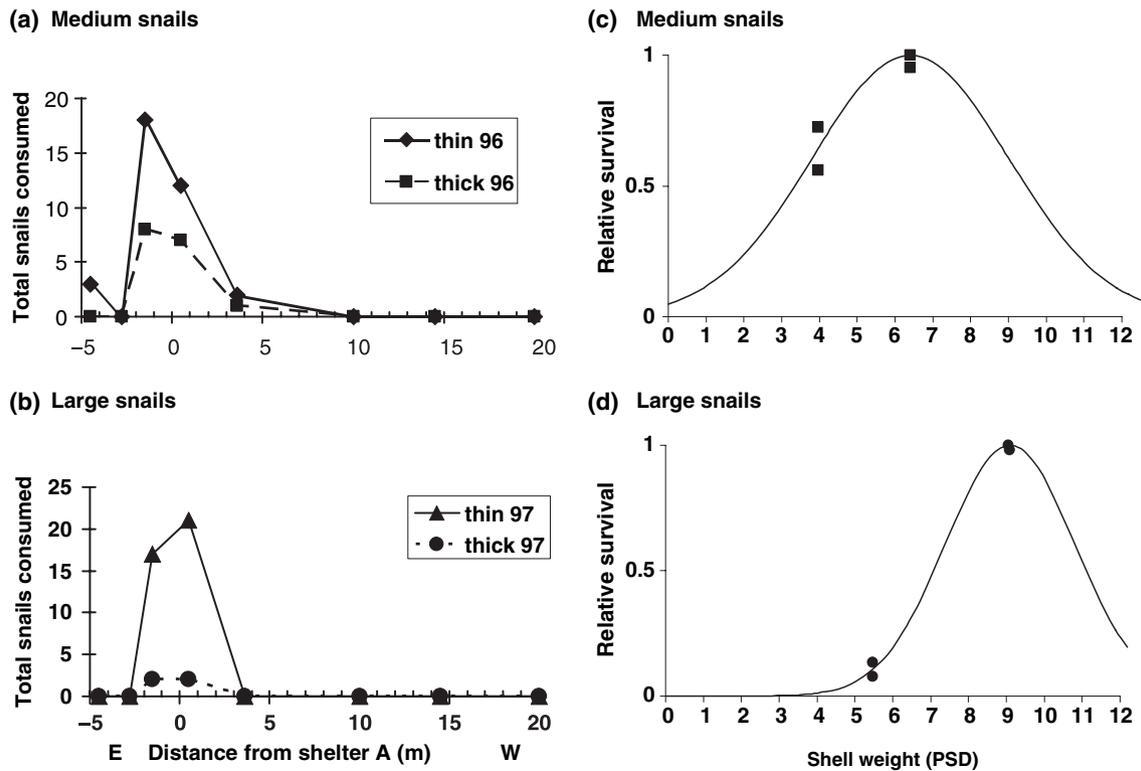


Fig. 1 Predation rate on tethered thick-shelled (*Littorina sitkana*) and thin-shelled (*L. subrotundata*) snails tethered at different distances from shelter complexes A at 0.0 m and B at 1.5 m E (Fig. S1; Table S2). Relative monthly survival was used to estimate the width, ω_c , of a Gaussian fitness function by fitting curves with a fixed optimum, θ_c , using nonlinear regression. (a) Medium size class of snails from 1996; (b) large size class of snails from 1997; (c) fitted curve ($r^2 = 0.876$) for 1996; (d) fitted curve ($r^2 = 0.997$) for 1997.

to the shelters (Fig. 1a,b). Large crabs were observed to evict smaller crabs introduced into their shelters. The crabs were especially active during night low tides and were not active when the shelters were submerged.

Fitness function

The shift in the optimum phenotype in the invaded populations, $\Delta\theta$, was greater for the large size class of snails than for the medium size class. A sample of the medium size class of sieved snails used in the 1996 tethering experiment had a predicted mean shell weight of 12.7 mg for *L. subrotundata* and 20.5 mg for *L. sitkana* (Table S3). The difference in their mean shell weights was 7.81 mg which, when divided by 3.2 mg/PSD, gave an estimate of $\Delta\theta = 2.44$ PSD for the medium size class of snails. The difference in the mean shell weights of the two species of snails used in the 1997 tethering experiment gave an estimate for the large size class of $\Delta\theta = 3.65$ PSD (Table S3). The estimate for the larger size class was larger than that for the medium size class because of greater positive allometry of shell thickness with shell length for *L. sitkana* than for *L. subrotundata* (Table S3).

The width of the fitness function in the invaded area, ω_c , was also greater for the large size class of tethered

snails than for the medium size class. Whereas the mortality of tethered snails was always significantly higher for *L. subrotundata* than for *L. sitkana* (Fig. 1a,b; Table S2), the difference in mortality between the two species was greater for the large size class used in 1997 (Fig. 1a) than for the medium size class used in 1996 (Fig. 1b). This difference in relative mortality resulted in two different estimates for the SD for the fitness function: $\omega_c = 2.60$ PSD for the medium size class of snails (Fig. 1c) and $\omega_c = 1.71$ PSD (Fig. 1d) for the large size class. The larger values of $\Delta\theta$ and ω_c for the large size class than for the medium size class indicate that the difference in the optimum was greater and the width of the fitness function was narrower. Therefore, when the mean phenotype of the core populations was not yet at the new optimum, both directional and stabilizing selection were stronger for the large size class than for the medium size class.

The laboratory predation experiments corroborated the tethering experiments in that the difference in mortality rates between the two species was greater for the large size class than for the medium size class. When offered *L. subrotundata*, *L. sitkana* and *L. scutulata/plena* together, shore crabs preferred the thin-shelled species to the two

thicker shelled species and this preference was stronger for the large size class of snails than for the medium size class (Supplementary Appendix S1).

Migration rates

The average along-shore distance that a tagged snail moved during its life time was large relative to the 19-m-long study area. If we assume a multiple stepping-stone model for a single generation, then the negative binomial dispersal kernel for the tagged snails from the 3.6 m W release point resulted in estimates of 10% moving 1 m, 7% moving 2 m, 5% moving 3 m, 3% moving 4 m, 2% moving 5 m, 1% moving 6 m, 1% moving 7 m and 0% moving 8 m in each of two directions (Table 2). The direct estimates of SD of lifetime dispersal displacements, σ , from recapturing snails with marked shells, ranged from 1.6 to 5.8 m (Table 2).

These direct estimates were similar in magnitude to the indirect estimate of $\sigma = 1.8$ m from the microsatellite data (Table S4). The slope of the regression of pairwise $F_{st}/(1 - F_{st})$ values vs. distance was $b = 0.0000495 \text{ m}^{-1}$ for Nudibranch site. If the census population size, N is assumed to be a good estimate of effective population size, N_e , then $N_e = 1615 \text{ m}^{-1}$ for a strip of 1 m wide (Supplementary Appendix S1). Since $1/b = 4N_e\sigma^2$ we obtained an estimate of $\sigma = 1.77$ m. The positive F_{is} values (Table S4) suggest that significant inbreeding occurs at the transect level. Therefore, N_e could be smaller than N_c which would increase this indirect estimate of σ and make it even closer to our direct estimate.

Other parameters

The size of the core area, k , where predation of tethered snails by the crabs had occurred represents about 4/19 of the 19-m-long study area at Nudibranch Point (Supplementary Appendix S1). We also found that the per capita fecundity of *L. subrotundata* did not increase significantly with the size of the female ($r = 0.464$, $P = 0.129$) and averaged 66.1 eggs per clutch (Supplementary Appendix S1).

Analytical model of cline

When Slatkin's (1978) analytical model for the medium size class of snails was parameterized with the direct migration estimate of $\sigma = 5.8$ m per generation, the heritability estimate, $h^2 = 0.3$, the standardized phenotypic variance, $V_z = 1.0$, and the SD of the fitness function for the medium size class of snails, $\omega = 2.60$ PSD (Table 1) it predicted a characteristic length, $l_c = 29.5$ m. If 1 m of the shore is equated to one model population, this suggests the core size must be at least 30 populations long before a detectable step cline will be formed. When the same calculation was done for the large size class of snails so that $\omega = 1.71$ PSD then the characteristic length, $l_c = 21.0$ m.

Finite locus multipopulation model

Large snails

It was only important to monitor demography for the large size class of snails when selection was unrealistically intense. There was no drop in population size when the finite locus model was parameterized for the large snails (Table 1) yet only partial adaptation occurred (38% at equilibrium) because selection was too weak to counteract the high multistep migration (Table 3). Less biologically realistic runs using intense selection ($\omega_c = 0.551$ PSD) allowed almost complete adaptation, despite high migration ($\sigma \approx 5.8$ m); however, the core populations went temporarily extinct (Fig. S2; Table 3). When the selection was even more intense ($\omega_c < 0.100$ PSD, $\sigma \approx 1.41$ m) no adaptation occurred because the core populations were converted to permanent sink populations (E.G. Boulding, unpublished data).

Medium snails

It was never important to monitor demography when the model was parameterized with values for the medium size class of snails because the population size never

Table 3 Finite locus model parameterized for the large size class of snails showing the effect of selection intensity, ω_c , and the core size, k , on the evolution of the mean shell thickness, \bar{z} , in invaded population 14, after 10 and after 100 generations.

ω_c in invaded pops	Core size, k	\bar{z} (SD)*
1.71 at 10	2/19	0.844 (0.0241)
1.71 at 100	2/19	0.884 (0.0167)
1.71 at 10	4/19	1.23 (0.0513)
1.71 at 100	4/19	1.41 (0.0279)
1.71 at 10	6/19	1.56 (0.7231)
1.71 at 100	6/19	1.92 (0.0114)
0.551 at 10	2/19	3.20† (0.0239)
0.551 at 100	2/19	3.27 (0.0251)
0.551 at 10	4/19	0.908‡ (0.3170)
0.551 at 100	4/19	3.37 (0.0089)
0.551 at 10	6/19	0.182§ (0.4070)
0.551 at 100	6/19	3.46 (0.0148)

\bar{z} , ω_b and ω_c are in phenotypic standard deviations (PSD). Initially for all populations the heritability, $h_i^2 = 0.3$, $\theta_i = 0.0$ PSD and $\omega_b = 1.0$ PSD. Two, four or six out of 19 populations were in the core area that was invaded by predators. After invasion the amount the optimum, was shifted $\Delta\theta$, was 3.65 PSD, and the width of the fitness function, ω_c , and became 1.71 PSD. All populations remained at carrying capacity unless otherwise noted. Means in bold font are from run using most biologically realistic parameters estimated from field experiment (Table 1).

*Mean and SD of core population 14 for generations 10–14, and generations 477–481 respectively.

†All core populations dropped below 20 individuals then recovered back up to carrying capacity by generation 5–6.

‡All core populations dropped below 0 individuals then recovered to carrying capacity by generations 88–325 ($N = 10$).

§All core populations dropped below 0 individuals then recovered to carrying capacity by generations 9–194 ($N = 10$).

Table 4 Finite locus model parameterized for the medium size class of snails showing the effect of selection intensity, ω_c , and the core size, k , on the evolution of the mean shell thickness, \bar{z} , in population 14, after 10 and 100 after generations.

ω_c in invaded pops	Core size, k	\bar{z} (SD)*
2.60 at 10	2/19	0.260 (0.0141)
2.60 at 100	2/19	0.280 (0.0141)
2.60 at 10	4/19	0.384 (0.0230)
2.60 at 100	4/19	0.422 (0.0148)
2.60 at 10	6/19	0.504 (0.0498)
2.60 at 100	6/19	0.638 (0.0130)
1.10 at 10	2/19	0.308 (0.00837)
1.10 at 100	2/19	0.346 (0.0195)
1.10 at 10	4/19	1.36 (0.0249)
1.10 at 100	4/19	1.38 (0.0148)
1.10 at 10	6/19	1.56 (0.0536)
1.10 at 100	6/19	1.70 (0.0122)

\bar{z} (standard deviation: SD), ω_b and ω_c are in phenotypic standard deviations (PSD). Migration is multi-step (Table 1). Initially for all populations the heritability, $h^2 = 0.3$, $\theta_i = 0.0$ PSD and $\omega_b = 1.0$ PSD. Two, four or six out of 19 populations were in the core area that was invaded by predators. After invasion the amount the optimum, was shifted, $\Delta\theta$, was 2.44 PSD, and the width of the fitness function, ω_c , became 2.60 PSD. All population remained at carrying capacity. Means in bold font are from run using most biologically realistic parameters estimated from field experiment (Table 1).

*Mean and SD of population 14 for generations 10–14 and generations 100–104 respectively.

dropped below carrying capacity. There was no reduction in population size using parameter values for the medium snails but only partial adaptation occurred (17% at equilibrium; Table 4). As expected, the lower estimate of migration from the 2.8 m E transect constrained local adaptation less than the higher migration estimate from the 3.6 m W transect but adaptation was

still incomplete (Fig. S3). Complete adaptation occurred only under the most intense selection ($\omega_c = 1.10$ PSD), the lowest migration ($\sigma \approx 1.09$ m), and the largest core size ($k = 6$) that was used (E.G. Boulding, unpublished data).

Core size

The most interesting general result was that increasing the number of the populations in the core from two to six almost doubled the amount of local adaptation shown by the populations at equilibrium but increased the time they needed to achieve it (Table 4). Adaptation was greater because when the core size was six most of the immigrants coming into populations 13 or 14 were from adjacent outer core populations that had experienced selection for the local environment. By contrast, when the core size was only two then all the immigrants entering population 13 or 14 from one side were from large populations that were not adapted to crab predation. This swamping effect was even stronger if the core populations declined below the carrying capacity because the number of emigrants was a fixed proportion of the total population size. Thus the total number of emigrants exported was larger for a large peripheral population whereas its mean phenotype was relatively unaffected by a few immigrants from a small, maladapted core population.

Comparison with models

There was good agreement between the amount of evolution of shell thickness predicted by the finite locus model and the increase in shell thickness observed near the crab shelters 10 years after the experiment began (Table S1; Table 5). The average difference in shell thickness at the two sites for wild snails collected near and far from the experimental crab shelters was 0.303 PSD (95%

Table 5 Validation of parameterized finite locus model results using experimental results from Dalziel & Boulding (2005).

Source	Generations of evolution	Mean response to selection of mean shell weight (PSD)	Standard error of response
Slatkin's (1978) cline model	Equilibrium	Much less than 1*	n/a
Finite locus model: medium size class snails†	10	0.384	0.0118‡
Finite locus model: large size class snails§	10	1.23	0.0317‡
Field-collected (near–far)¶	10–20	0.303	0.127**

Response to selection (increase) in shell weight in 'core invaded populations' shown in phenotypic standard deviations (PSD) to facilitate comparison of model results with those from the field experiment.

*M. Slatkin (personal communication).

†Shift in optimum $\Delta\theta$ was 2.44 PSD from an initial optimum of 0.0 PSD towards the new optimum of 2.44, $m =$ multiple step (see text), $h^2 = 0.3$, core size = 4 and $\omega_c = 2.60$ for this simulation for the medium size class of *Littorina subrotundata* (Table 3).

‡Back-transformed averaged standard error from square root-transformed model output after 500 parametric bootstrappings of five input parameters. See text, Fig. S3, and Table S6 in Supplementary Appendix S2.

§Shift in optimum $\Delta\theta$ was 3.65 PSD from an initial optimum of 0.0 PSD towards the new optimum of 3.65, $m =$ multiple step (see text), $h^2 = 0.3$, core size = 4 and $\omega_c = 1.71$ for this simulation for the large size class of *L. subrotundata* (Table 4).

¶The difference in mean shell weight between wild *L. subrotundata* collected near (< 1 m) to the experimental crab shelters and those collected farther away (> 5 m). Shell weight from Dalziel & Boulding (2005) was converted from milligrams to PSD by dividing by 3.2 mg per PSD. See Table S1 for dates of shelter construction.

**Root mean standard deviation was divided by square root of the sample size then divided by 3.2 mg per PSD.

CL: 0.249–0.552) (Table 5). This was similar to the increase in shell thickness of 0.384 PSD (95% CL: 0.361–0.407) that was predicted by the finite locus model parameterized for the medium size class of snails (Table 5). However, this was lower than the increase in shell thickness of 1.23 PSD (95% CL: 1.17–1.29) predicted by the finite locus model parameterized for the large size class of snails (Table 5).

When parameterized for the medium size class of snails, Slatkin's (1978) analytical model predicted no detectable adaptation until the length of the region invaded by the crabs was at least 30 m long. This differed from the finite locus model which predicted local adaptation of 0.422 PSD within a distance of only 4 m ($k = 4$; Table 4). It also differed from the field observations which showed a significant increase in shell thickness near the shelters even though the length of the region with increased predation was only 4 m (Table 5). The lack of local adaptation to short gradients in predation by Slatkin's (1978) model was not a result of its assumption that $\omega_c = \omega_b = 2.6$ PSD. When we re-ran the finite locus model with $\omega_c = \omega_b = 2.6$ PSD, the amount of adaptation it predicted at equilibrium with $k = 4$ increased to 0.756 PSD.

Discussion

Our finite locus model differed from Slatkin's (1978) model in predicting local adaptation over a distance less than his characteristic length. Slatkin's (1978) parameterized model predicted that shell thickness could not significantly respond to the 4-m pocket of increased predation created by our field experiment because its length was less than 30 m. In his 1978 model, 'the mean of the character does respond to a patch that is smaller than the characteristic length, but the response is much less than the SD of the character and hence not detectable in practice' (M. Slatkin, personal communication). By contrast, when parameterized for our field experiment, our finite locus model for the medium size class of snails predicted partial adaptation (0.422 PSD at equilibrium) to the introduced crabs within a distance of only 4 m. Further, parameterization of our model for the large size class of snails predicted partial but more substantial adaptation (1.41 PSD) to the crabs within a length of 4.0 m.

The discrepancy between the predictions of Slatkin's (1978) model and those of our finite locus model most likely has to do with our field experiment's violation of his assumptions rather than problems with either model. He specified that his model could only apply to systems where: (1) all parameters except the optimum were the same everywhere; (2) the range of optima was significantly smaller than one PSD; and (3) the ratio of the phenotypic variance to the variance of the fitness function was small so that optimizing selection was weak. For the medium and particularly for the large size

class of snails all three of these assumptions were violated.

The predictions of models of clines in quantitative traits differ substantially from those for single locus traits because changes in the mean are decoupled from changes in the variance (Slatkin, 1978). Slatkin argued that strong optimizing selection could result in clines evolving within distances shorter than his characteristic length because immigration of individuals from locations where the optimum is different increases the additive genetic variance in the middle of the cline. Barton (1999) showed that linkage disequilibrium can be the main cause of an increase in the additive genetic variance under spatially varying strong selection and that the increase will be concentrated where the gradient in trait means is steepest. Tufto (2000) found that there was significantly more local adaptation in an infinitesimal model that included this source of positive linkage disequilibrium than in a version of the model that did not. Barton (2001) relaxed the assumption made by the cline model of Kirkpatrick and Barton (1997) of constant additive genetic variance and found that he was more likely to get adaptation at the species' range margin. In our finite locus model linkage disequilibrium created by immigrants from populations where the optimum was different likely caused an increase in the local additive genetic variance. An increase in the genetic variance above that expected from the initial heritability would result in the evolution of a step cline over distances of less than 4 m. This mechanism would be expected to act similarly in our experimental snail populations where alleles increasing shell thickness would be expected to be in positive linkage disequilibrium because of the steep gradient in predation.

Our field data support the predictions of our finite locus model in showing a small but detectable cline in shell thickness near the shelters after only 10 years without any major drop in population size. The observed difference in shell thickness between wild snails collected close to the crab shelters and those collected farther away (Dalziel & Boulding, 2005) was similar to that predicted by our model. Further, juvenile snails collected from near the main shelter at Prasiola Point developed consistently heavier shells when grown in a common environment than did snails collected from farther away, suggesting that the observed difference has a genetic basis (Dalziel & Boulding, 2005).

Other field studies have also documented only partial adaptation to microenvironmental gradients in selection when gene flow was high. Jain & Bradshaw (1966) reviewed data on clines in heavy metal tolerance in grass species and showed several cases of plant populations maladapted to clean soil because of gene flow from plant populations on contaminated soils. Dhondt *et al.* (1990) found mean clutch size in some bird populations were not at the optimum for the local environment, which he

attributed to gene flow from regions where the optimal clutch size was different.

Our tethering experiment confirms previous work conducted in the laboratory and at a larger spatial scale in the field (reviewed by Boulding *et al.*, 1999) showing that increased shell thickness increases snail survival in the presence of shore crabs. We found that thick-shelled *L. sitkana* had a higher survival than the thin-shelled species, *L. subrotundata* when tethered in areas where shore crab predation rates were high, and that both species survived equally well where predation rates were low. Thicker littorinid shells have thicker lips that increase their resistance to small shore crabs trying to peel open the shell (Boulding & Van Alstyne, 1993; Behrens Yamada & Boulding, 1998). The shell fragments we recovered allowed us to identify the predator as the transplanted shore crabs because other possible shell-crushing predators, such as subtidal crabs and fish, cannot forage effectively in the intertidal zone of wave-exposed shores (Boulding *et al.*, 1999). Tethering artefacts were likely minimal because these slow-moving snails graze, seek refuges and even copulate while tethered (Boulding *et al.*, 1999). Further, our laboratory experiments support our finding in the tethering experiments that shore crabs strongly prefer the thin-shelled species when offered snails of the large size class. Both our laboratory and tethering experiments showed that this shore crab's preference for the thinner shelled species was less marked when offered snails of the medium size class because the difference in shell thickness was less. Shore crabs also show a significant preference for thinner shelled *L. subrotundata* over thicker shelled *L. subrotundata* of the same size in laboratory experiments but this within-species preference is too small to be easily detected in short-term field tethering experiments (D. Pakes and E.G. Boulding, unpublished).

Our transplanting of predatory crabs into a prey population where they are usually rare could have at least three effects other than the evolution of thicker shells by *L. subrotundata* near the crab shelters. First, *L. subrotundata* could show an ecophenotypic increase in shell thickness in response to effluent from the shore crabs (Trussell, 1996; Behrens Yamada *et al.*, 1998). Second, if predation is intense then competitive replacement of thin-shelled *L. subrotundata* by the thicker shelled *L. scutulata* or *L. plena* could occur. Third, *L. subrotundata* could migrate out of the areas near the crab refuges (Behrens Yamada *et al.*, 1998). The increased shell thickness of snails near the shelters does have an ecophenotypic component but the response persists in a common environment indicating that it also has a genetic component (Dalziel & Boulding, 2005; Supplementary Appendix S1). Further, our permanent quadrat data suggest that neither replacement nor directional migration is occurring (Supplementary Appendix S1; E.G. Boulding, unpublished data).

Our work shows that including demography in models can be important when selection is strong, as it often is in systems selected for study by evolutionary ecologists. Most interestingly, we found that if we scaled the strong stabilizing selection we measured in our tethering experiments for the large size class of snails continued over a 180-day period, temporary extinction of the core populations was predicted. Thus in a model with demography, high migration can prevent local adaptation because very strong stabilizing selection will cause the local population to go extinct. This interpretation is consistent with existing empirical data showing that when populations experience a large change in their environment they often go extinct (e.g. Zaret & Paine, 1973; Atkinson, 1977; Clarke *et al.*, 1984; Reznick, 2001). The importance of including demography in quantitative genetic models has been previously noted in the literature (Pease *et al.*, 1989; Lynch & Lande, 1993; Gomulkiewicz & Holt, 1995; Kirkpatrick & Barton, 1997; Boulding & Hay, 2001). On the other hand, it was encouraging to find that including demography in the model was not important when the most biologically significant parameters were used because the population size never dropped below carrying capacity.

In our model, runs with a core size of six populations always showed more adaptation than simulations with core areas of two populations, especially when migration was high. This suggests that selection by crabs on the *L. subrotundata* populations near the crab shelters resulted in a smaller step cline in shell thickness than if the shelters had been built over a larger area. The importance of core size suggests that an invasion by a nonindigenous species of only one or two populations may be more likely to cause extinction through *migrational meltdown* than a more widespread invasion of a series of adjacent populations. Pease *et al.* (1989) considered migration and adaptation of a single population in response to a moving linear environmental gradient and found that the range of suitable habitat had to reach a minimum size before the species would persist.

The modelling results suggest that direct-developing *Littorina* species would be more likely to show local adaptation to predators when the crabs inhabit a larger area than when they inhabit a smaller area. Large genetic differences between populations for traits such as shell form, behaviour and life-history traits that have been described for other direct-developing *Littorina* ecotypes often seem to be associated with the presence and absence of crab predation in different regions of their habitat (reviewed by Reid, 1996; E.G. Boulding, personal observation). We know that such patterns of local genetic adaptation by *L. saxatilis* are convergent because shore crabs are restricted to the upper shore in Spain (Johannesson *et al.*, 1993), the lower shore in Britain (Wilding *et al.*, 2001) and protected shores in atidal Sweden (Janson, 1983). The results presented here show that the reason why the presence of shore crabs can have an

extremely localized effect on selection is that crabs are reluctant to forage on snails far from a refuge. Consequently, we would predict that crab-adapted ecotypes would likely evolve whenever the length of contiguous habitat occupied by the crabs is equal to the *characteristic length* for that particular predator–prey system.

Acknowledgments

We thank M. Slatkin for help with his analytical models and J. Felsenstein for early help with our finite locus model. We also thank C. Caruso, B. Dalziel, T. Lenormand, I. Olivieri, M. Ritchie and M.C. Whitlock for their suggestions for improving the manuscript, L. Kusumo and many short-term field assistants for technical assistance, the Director and staff of Bamfield Marine Sciences Centre for field support, and the Huu-ay-aht First Nation for access to our study sites. Financial assistance was provided by Natural Sciences and Engineering Research Council of Canada 'Discovery' grants, and an Ontario Premier's Research Excellence Award to E.G.B.

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Supplementary Material

The following supplementary material is available for this article:

Figure S1 Study site at Nudibranch Point.

Figure S2 Effect of more intense stabilizing selection and a larger shift in the optimum on the evolution of shell thickness.

Figure S3 Effect of increased migration on the evolution of shell thickness.

Figure S4 Predicted distribution of shell thickness from parametric bootstrapping of five input parameters.

Table S1 Details of construction of crab refuges.

Table S2 Summary of four tethering experiments.

Table S3 Shell weight differences between *L. subrotundata* and *L. sitkana*.

Table S4 Population genetic structure using hierarchical *F*-statistics.

Table S5 Population statistics for six microsatellite loci.

Table S6 Standard errors from bootstrapping five input parameters.

Appendix S1 Details of parameter estimation from field experiment.

Appendix S2 Details of finite locus model.

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Received 6 November 2006; revised 26 March 2007; accepted 27 March 2007