

ORIGINAL ARTICLE

Epistasis, phenotypic disequilibrium and contrasting associations with climate in the land snail *Theba pisana*

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Hotter conditions favour effectively unbanded (EUB) shells in the snail *Theba pisana*. *T. pisana* is also polymorphic for colour of the shell's apex, determined by a pair of alleles at a locus linked to the banding locus. Apex colour is epistatic to shell banding, such that banded snails with a dark apex have darker bands. Annual censuses over 22 years across an ecotone between a sheltered *Acacia* thicket and open dune vegetation showed a persistent association of both EUB shells and pale apex with the Open habitat. The parallel variation was due partly to strong phenotypic disequilibrium, as the combination of EUB with dark apex was rare. Nevertheless, in fully banded shells the frequency of pale apex was also higher in the Open habitat, confirming independent, parallel associations of the two contributors to paleness. Within the *Acacia* habitat, temporal variation of the frequencies of banding morphs was much greater than for apex colour, and EUB shells were associated with hotter summers. Consistent with its primary effect only on the very small snails, apex colour did not vary with summer conditions, but instead, higher frequencies of pale apices were associated with sunnier winters. The intensity of selection was lower on apex colour than shell banding, due partly to the constraint of phenotypic disequilibrium. The shell traits in *T. pisana* are an example of complex responses to climatic variation, in which phenotypic disequilibrium constrains evolution of apex colour, but separate mechanisms of selection are evident.

Heredity (2012) **108**, 229–235; doi:10.1038/hdy.2011.62; published online 3 August 2011

Keywords: adaptation; ecotone; selection coefficient; shell polymorphism; temperature; temporal variation

INTRODUCTION

Adaptation to significant environmental variation is likely to involve many traits, and the complexity of genetic interactions can lead to linkage disequilibrium and constraints to independent evolution of individual traits (for example, Lande, 1984; Blows, 2007; Walsh and Blows, 2009). Visible polymorphisms, such as colour polymorphisms, have had a significant role in the study of adaptation, because they are readily accessible and often have clear adaptive significance. They are also valuable for examining the complexity of adaptation, because they are frequently correlated with other traits (McKinnon and Pierotti, 2010). Shell polymorphisms in snails are useful models for studying the complexity of adaptation, because likely mechanisms of selection are often clear in terms of selective predation or climatic selection (Jones *et al.*, 1977; Clarke *et al.*, 1978; Cain, 1983; Vermeij, 1993), allowing predictions of responses to changes in habitat or climate. Examples of multiple traits associated with significant environmental gradients include colour and banding in the land snail *Cepaea nemoralis* (see Jones *et al.*, 1977; Cook, 2005; Ozgo, 2011), colour and shape in the littorine *Bembicium vittatum* (Johnson and Black, 2008), and shape and molecular markers in *Littorina saxatilis* (Grahame *et al.*, 2006; Galindo *et al.*, 2010).

The land snail *Theba pisana* is genetically polymorphic for shell banding, and variation of morph frequencies indicates adaptation to local climate: snails with paler, unbanded shells are favoured in exposed habitats (Johnson, 1980, 2011a; Heller, 1981) and in hot, sunny summers (Johnson, 2011a), and they select more exposed

aestivation sites in summer than their banded counterparts (Johnson, 1981; Hazel and Johnson, 1990). The banding polymorphism is complex, and effectively unbanded (EUB) shells may be either genetically unbanded or banded with genetic suppression of bands on the upper portion of the shell (Cain, 1984; Cowie, 1984). In response to contrasting habitats, the frequency of EUB in banded snails is correlated with the frequency of genetically unbanded in the local population, indicating that multiple mechanisms of producing pale shells are important in adaptation to exposed habitats (Johnson, 1980). On a broader scale, adaptation of *T. pisana* to climate also involves body colour (Cowie, 1990). Inter-specific comparisons including *T. pisana* indicate that the inducibility of heat-shock proteins is also associated with variability of shell pigmentation (Kohler *et al.*, 2009), highlighting the complexity of adaptation to climatic conditions.

Another trait that might be expected to contribute to adaptation to microclimate in *T. pisana* is apex colour (pigmentation of the first whorl of the embryonic shell), variation of which is determined by two alleles at a locus linked to the main banding locus, with pale dominant to dark (Cain, 1984; Cowie, 1984). There are two important differences between apex colour and shell banding, however. First, the direct effects of apex colour are much less than for banding, because they are restricted to the protoconch (the shell at hatching), so we might expect less intense selection. Second, however, there is a greater effect on young juveniles, in which apex colour is the dominant source of pigmentation, so we might expect different effects of seasons on apex

colour than on banding. Furthermore, strong phenotypic disequilibrium has been found between apex colour and shell banding (Cowie, 1984), and there appears to be an epistatic effect of apex colour on the intensity of shell banding, which together could both enhance parallel adaptation and restrict the independent evolution of the two traits.

In the population of *T. pisana* at Bold Park, Perth, Western Australia, annual sampling since 1977 along a transect through a dense *Acacia* thicket and adjacent open, low vegetation has shown the persistence of abrupt changes in banding morph frequencies associated with the contrasting habitats (Johnson, 2011a). The long-term annual censuses of the Bold Park population showed increases of the proportion of EUB shells associated with hotter, drier summers, and provided 33 estimates of annual selection coefficients, within a clear adaptive perspective, which showed that selection can be very strong ($s=0.5$), but is often weak. Reviews of measurements of selection in nature have emphasized the scarcity of such continuous, long-term studies, especially of invertebrates, despite their value in determining the intensity and the variability of selection under natural conditions (Hendry and Kinnison, 1999; Hoekstra *et al.*, 2001; Hereford *et al.*, 2004; Siepielski *et al.*, 2009). The classic example of directional selection on industrial melanism in the moth *Biston betularia* (for example, Cook and Turner, 2008) and the analysis of fluctuating selection on shell banding in *T. pisana* over 34 years at Bold Park (Johnson, 2011a) provide the most thorough temporal studies of selection in polymorphic invertebrates in natural populations.

The present study extends the analysis of the population of *T. pisana* at Bold Park to examine spatial and temporal variation in apex colour. Apex colour has been scored in this population since 1989, providing a sequence of 22 consecutive years. In this paper I (1) document epistasis between apex colour and shell banding; (2) test for phenotypic disequilibrium between the two traits; (3) test for parallel associations with habitat as an indication of common adaptation; (4) use the temporal series to test for contrasting seasonal effects on frequencies of apex colour morphs and shell banding morphs and (5) compare intensities of natural selection on the two traits, to test the expectation that selection is less on apex colour than on banding.

MATERIALS AND METHODS

Study area and sampling

The study site is an area of coastal dunes in the southwest corner of Bold Park (31° 57' 06" S, 115° 45' 21" E), in City Beach, Perth, where abundance and the banding polymorphism in *T. pisana* have been studied since 1977 (Johnson, 2011a,b). This Mediterranean species was introduced to Western Australia in the 1890s, and the population at Bold Park was well established by the time the study began. Samples for the present analysis were collected each year from 1989 to 2010, along a transect across the transition between a dense thicket of *Acacia rostellifera* bushes and an area of low, sparse dune vegetation (areas Acacia-I and Open-I in Johnson, 1980). The transect comprised contiguous 5×5-m quadrats, except in the western portion of the Open habitat, where the dune vegetation was patchy and shifted between years. The length of the transect sampled varied among years, but included ≥85 m into the Open habitat in all years except 1992 (30 m) and ≥35 m into the Acacia habitat. Within each quadrat, three or four people systematically searched the ground and the vegetation, attempting to collect all snails ≥10 mm in diameter (=adults and near-adults). The snails were scored for shell banding and apex colour, and returned to their quadrats of origin within 2 weeks of collection, to have minimal impact on the population.

At each census, the maximum height of vegetation was measured in each 1-m interval along the transect, and the average was recorded for each quadrat.

Quadrats with ≥60% coverage by bushes were classified as Acacia habitat, whereas those with ≤40% were classified as Open. In 12 of the years, the quadrat at the ecotone had intermediate cover and was excluded from comparisons between habitats. Samples were collected in autumn (late March through May, 16 years) or winter (July to early August, 6 years). Near the habitat boundary, seasonal migration of many snails to and from aestivation sites causes local changes in morph frequencies in the Open habitat, within 15–20 m of the Acacia, and in the Acacia quadrat adjacent to the Open, but such seasonal variation within the rest of the Acacia habitat is small (Johnson, 1981).

Shell polymorphism

The shell of *T. pisana* is white, with varying numbers and intensities of brown bands, determined primarily by alleles at the banding locus, but with modifiers at other loci (Cain, 1984; Cowie, 1984). As in previous studies of the Bold Park population (Johnson, 1980, 1981, 2011a; Hazel and Johnson, 1990), shells were classified simply as either EUB (in which bands are absent from the upper half of each whorl) or fully banded (FB, with some banding on the dorsal portion of the shell). The last 90° of the outermost whorl were ignored, because pigment is sometimes present in the newly formed shell, even of genetically unbanded snails (Cain, 1984). In addition to the variation in banding, the colour of the shell apex (the protoconch, or newly hatched shell) is either pale (various shades of grey-brown) or dark (very dark purple-black on at least a portion of the apex), depending on alleles at a single locus, with pale dominant to dark. This locus is linked to the main banding locus (Cain, 1984; Cowie, 1984); from the three informative matings reported by Cain (1984, Table 10), only 21 of 385 offspring (5.5%) were recombinants. Combining the two traits, the shells were thus classified into four phenotypes: EUB Pale, EUB Dark, FB Pale and FB Dark (Figure 1).

When scoring the shells, it was evident that there was epistasis, in which banding was often more intense in the FB shells with a dark apex than in those with a pale apex. To confirm this interaction, the brightness of shells was measured in a random sample of 123 adults (≥12 mm diameter) collected within a radius of 10 m in the Acacia habitat in September 2009. In this population, the snails live up to 2 years, and there is a post-reproductive winter die-off of adults in July and August, so adults collected in September would have matured after the reproductive season and thus had experienced one summer. They were collected approximately 50–70 m from the Open habitat. In a mark–recapture study at this site, 75 of 105 recaptured snails had moved ≤5 m from February to July, and only one moved more than 25 m, with an average of 6.7 m (Johnson, 1981). Even the rate of expansion into unoccupied,

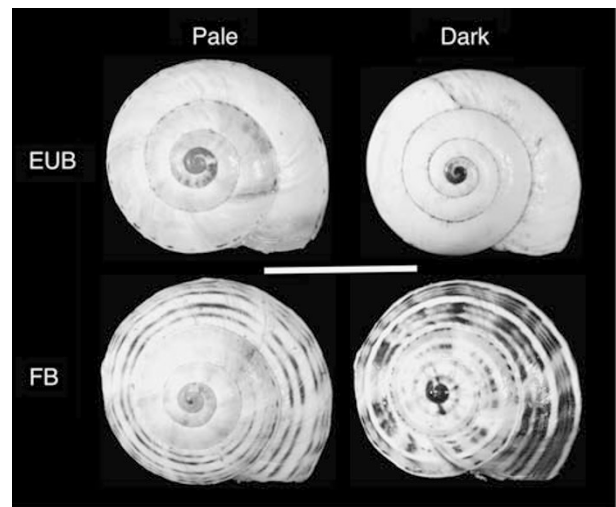


Figure 1 Examples of the combined phenotypes of apex colour and shell banding, representing average intensities of pigmentation for each category. White bar: 1 cm.

available habitat averaged only 21 m per year (Johnson and Black, 1979), so all adults collected in September 2009 had almost certainly spent their entire development in the Acacia habitat. This uniformity minimized any direct effects of habitat or age on variation in the intensity of banding. The apical (dorsal) portion of each shell was photographed under identical magnification and fibre optic lighting, using an Olympus DP70 digital camera mounted on an Olympus SZ61 dissecting microscope, with white balance activated. The brightness of each shell was measured digitally using ImageJ (Abramoff *et al.*, 2004), on a scale of 0 (black) to 255 (white). The last 90° of the final whorl were excluded. The apex was also excluded to remove any direct effect of apex colour on the overall pigmentation of the shell.

Analysis

In the annual collections, the proportions of the four morphs (EUB Pale, EUB Dark, FB Pale and FB Dark) were calculated for each quadrat in each year, and analyses were based on the means among quadrats. The data for each quadrat are available in Dryad (doi:10.5061/dryad.q318s). Quadrats with fewer than 10 snails were excluded from all analyses (4 of 370 Acacia quadrat samples and 29 of 176 Open samples). The average sizes of the remaining samples were 195 ± 162 (s.d.) in the Acacia and 82 ± 94 (s.d.) in the Open habitat. To summarize variation along the transect, morph frequencies were averaged across years for each 5-m interval. Over the period of this study, the Acacia habitat spread 15 m into the Open. The means were therefore taken for intervals relative to the habitat boundary, to allow comparison between the habitats. Only means based on at least 3 years were included.

Analysis of temporal variation was restricted to the Acacia habitat, because lower abundance in the Open meant that errors of estimation of individual samples were much larger, and there were fewer years with enough quadrats with at least 10 snails. For each year, the morph frequencies in the Acacia quadrats were averaged, to provide the mean for that year. Many snails from the adjacent Open area move to the *Acacia* bushes at the boundary for summer aestivation (Johnson, 1981), seasonally inflating the local frequency of EUB. Because 16 of the 22 annual censuses were in autumn, the Acacia quadrat closest to the Open habitat was excluded from the temporal analysis, to get more reliable estimates of morph frequencies in the Acacia habitat. Restriction to the Acacia habitat allowed analysis of temporal variation, without the confounding effects of habitat.

The intensity of natural selection was estimated annually, based on changes in the frequency of banding and apex colour morphs in the Acacia habitat from one year to the next. The yearly sampling interval is within a generation (1–2 years), so the selection required to cause the changes in the frequencies of a morph was estimated simply as $s = (1 - (P_0/P_1)) / (1 - P_0)$, where P_0 is the initial frequency of the favoured morph and P_1 is the frequency in the following year (Johnson, 2011a). To allow comparisons of intensity of selection, regardless of direction, the estimates were expressed as absolute values. Because absolute errors of estimation are always positive, estimates of the absolute value of selection are biased upwards (Hereford *et al.*, 2004). The bias of each annual estimate was removed by removing the effects of sampling error, as detailed previously (Johnson, 2011a; details in Supplementary Table 1).

Over the 34-year period 1977–2010, higher frequencies of EUB were associated with hotter, sunnier and drier summers, but not with weather variables in other seasons (Johnson, 2011a). Because apex colour has its main effect on baby snails, I expected a different association with weather, in which conditions in winter and possibly autumn, the period of reproduction and early growth, would be more important. To test the predicted associations of morph frequencies with climatic conditions, weather observations were obtained for Perth from March 1987 through February 2010 (Australian Bureau of Meteorology), the period that could have direct effects on the present set of samples. For each season (summer: December–February; autumn: March–May; winter: June–August; spring: September–November) four weather variables were recorded: average daily maximum temperature; mean number of hours of sunshine per day; number of days with detectable rainfall and amount of rain. For summer, number of days with maximum temperature $\geq 30^\circ\text{C}$ and number of days with maximum $\geq 40^\circ\text{C}$ were also recorded. Principal-components analyses were used to find composite measures of weather conditions in each season. Associations of morph frequencies with each

composite seasonal weather variable were tested by Pearson correlation. Further tests of association were made for the individual weather variables, because these can provide a clearer understanding of the associations with the composite variables (Johnson, 2011b). The snails were up to 2 years old at the time of sampling, so the independent variables were the averages of each weather variable over the 2 years preceding sampling. Although these analyses were performed previously over the 34-year period for shell banding (Johnson, 2011a), they were repeated for the 22-year period, to allow direct comparisons with apex colour.

RESULTS

Interaction of banding and apex colour

Measurements of brightness confirmed epistasis between apex colour and shell banding, such that FB Dark shells were darker, on average, than FB Pale, although distributions of scores were broad and overlapping (Figure 2). Mean (\pm s.e.) brightness scores (excluding the apex) were 186.3 ± 2.3 for FB Dark, compared with 207.2 ± 1.3 for FB

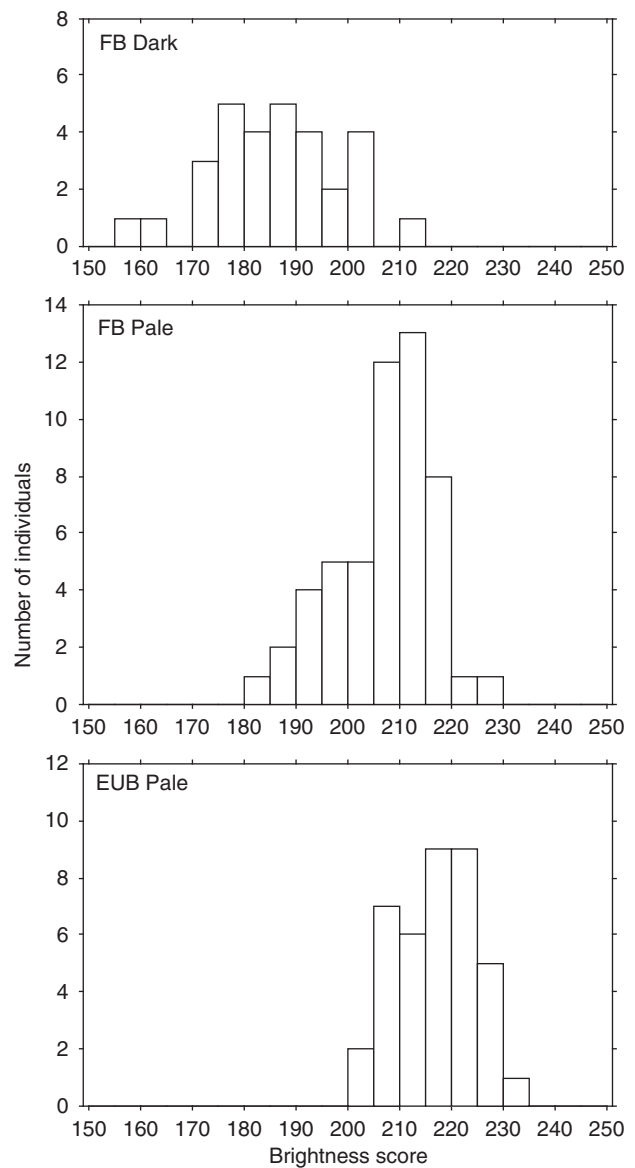


Figure 2 Frequency distributions of average brightness scores for three combinations of shell banding and apex colour: FB Dark, FB Pale and EUB Pale. The apex was excluded from these measurements.

Pale and 216.5 ± 1.2 for EUB Pale ($F_{2,118}=84.927$, $P<0.0001$), indicating that FB Pale is an intermediate phenotype, closer in brightness to EUB Pale than to FB Dark. The two EUB Dark shells in the sample had a mean of 213.4, indistinguishable from the EUB Pale. The shells in Figure 1 are close to the mean brightness for each morph.

So few EUB Dark shells were examined because the combination was consistently rare throughout the entire study site. Of the 90 136 snails scored from 1989 to 2010, only 529 (0.59%) were EUB Dark. The other three combinations were all common: 36 148 EUB Pale (40.10%), 32 945 FB Pale (36.55%) and 20 414 FB Dark (22.65%).

Spatial and temporal variation

Over the 22 years, there was a persistent association of the frequencies of the three common morphs with habitat, whereby EUB Pale was at higher frequencies in the Open habitat than in the Acacia, and both FB Pale and FB Dark showed the reverse association (Figure 3). Based on the values in Figure 3, the mean (\pm s.e.) proportions of EUB Pale were

0.625 ± 0.012 in the Open habitat and 0.364 ± 0.012 in the Acacia ($t=14.966$, $df=39$, $P<0.0001$). For the darkest morph, FB Dark, the mean frequencies were 0.087 ± 0.007 in the Open habitat, compared with 0.242 ± 0.007 in the Acacia ($t=15.069$, $df=39$, $P<0.0001$). The intermediate combination, FB Pale, showed a less marked, but significant, association with habitat, with mean frequencies of 0.278 ± 0.011 in the Open habitat and 0.386 ± 0.007 in the Acacia ($t=9.029$, $df=39$, $P<0.0001$). The mean frequencies of the rare morph, EUB Dark, did not differ between the habitats.

Because of the extreme rarity of the EUB Dark phenotype, the frequencies of EUB Pale and FB Dark indicated clear associations of both apex colour and banding with habitat, as higher frequencies of both Pale and EUB occurred in the Open habitat. To test whether apex colour varied with habitat, independent of its disequilibrium with banding, the proportion of Pale was examined within FB shells. Only samples with ≥ 10 FB snails were used, and only quadrat means based on ≥ 3 years, so the portion of the transect that could be included extended only 60 m into the Open habitat. Nevertheless, a clear association of apex colour (within the banded snails) with habitat was evident (Figure 4, left), with mean frequencies of Pale within FB being 0.713 ± 0.020 in the Open, compared with 0.615 ± 0.005 in the Acacia ($t=7.82$, $df=30$, $P<0.0001$). There was a strong association between the frequency of Pale in FB and the frequency of EUB (Figure 4, right), confirming that these two contributors to paler shells varied in concert, independent of their phenotypic disequilibrium.

In contrast with the strong association of spatial variation of Pale in FB with EUB, the two traits showed very different patterns of change among years, within the Acacia habitat (Figure 5). Variation among years was statistically significant for both traits (F -tests, $P<0.0001$), but the frequencies of Pale in FB were negatively correlated with those of EUB ($r=-0.434$, $P=0.044$). The temporal variation was much less for Pale in FB ($s^2=0.001515$, coefficient of variation (CV)=6.36 for Pale in FB, compared with $s^2=0.003867$, CV=17.08 for EUB; comparison of variances, $F_{21,21}=2.55$, $P=0.019$), and it was even less for the total frequency of Pale ($s^2=0.000634$, CV=3.38). The estimated annual selection coefficients were less than 0.05 in most years, but in individual years were as high as 33% for banding, 15% for apex colour overall and 26% for apex colour within the banded

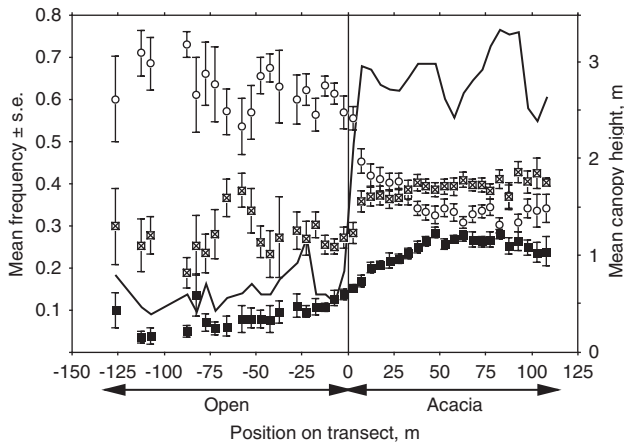


Figure 3 Mean (\pm s.e.) proportions of the three common morphs along the transect relative to the position of the habitat boundary (0 m, indicated by vertical line) over 22 years of sampling: EUB Pale (circles), FB Pale (squares with x) and FB Dark (filled squares). The solid line shows mean canopy height.

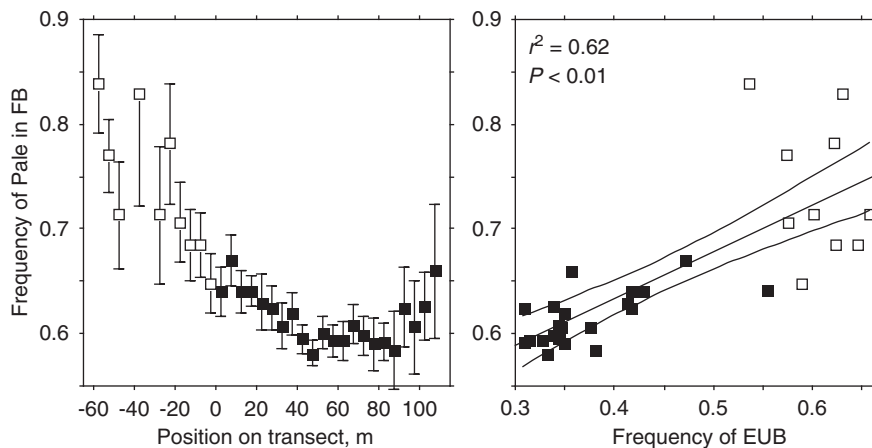


Figure 4 Left: Mean (\pm s.e.) proportions of pale apex within the FB snails along the transect relative to the position of the habitat boundary (0 m) over 22 years of sampling. The open symbols denote the Open habitat and the filled circles denote the Acacia habitat. Right: Regression (\pm 95% confidence interval) of these proportions of pale apex within the FB snails against the mean proportions of EUB shells in the same samples.

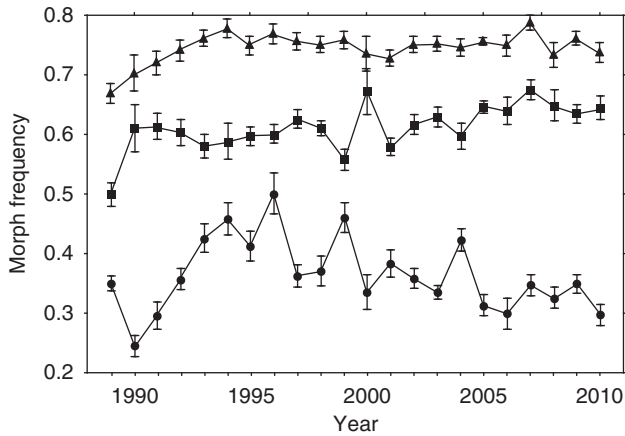


Figure 5 Mean (\pm s.e) proportions of EUB shells (circles), pale apices (triangles) and pale apices in FB snails (squares) over time in the Acacia habitat. Only means based on ≥ 3 quadrats with $n \geq 10$ snails are included.

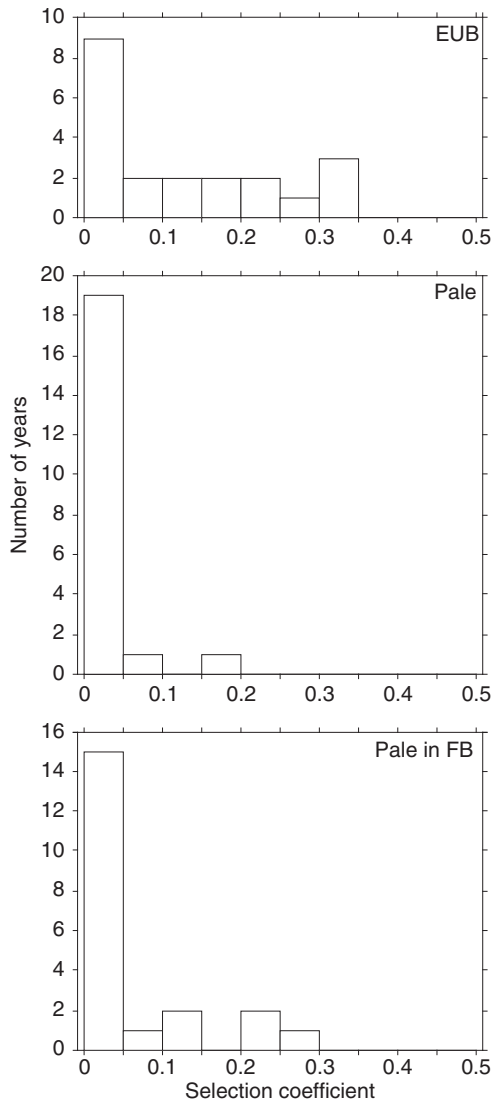


Figure 6 Frequency distribution of absolute annual selection coefficients, estimated from changes in morph frequencies in the Acacia habitat, for EUB, Pale and Pale in FB.

Table 1 Correlations of weather variables with principal components for each season, based on data from March 1987 to February 2010

Variable	Summer		Autumn		Winter	Spring
	PC1 (52.5)	PC2 (18.1)	PC1 (53.1)	PC2 (29.1)	PC1 (65.9)	PC1 (55.6)
Mean T_{max}	0.679	0.601	-0.104	0.904	0.427	0.833
Sunshine	0.864	-0.238	-0.855	-0.184	0.883	0.793
Rainfall	-0.735	0.540	0.767	0.152	-0.907	-0.554
Rain days	-0.866	0.134	0.891	0.093	-0.922	-0.772
Days $\geq 30^\circ\text{C}$	0.599	0.593	—	—	—	—
Days $\geq 40^\circ\text{C}$	0.489	-0.079	—	—	—	—

$r \geq 0.5$ in boldface. Percent variance represented by each component is shown in parentheses. Only components with eigenvalues ≥ 1 are included.

Table 2 Correlations of the annual mean frequencies of EUB and Pale in the Acacia habitat with weather variables over 22 years

Variable	EUB		Pale		Pale in FB	
	r	P	r	P	r	P
Principal components						
Summer PC1	+0.483	0.023				
Winter PC1					+0.457	0.033
Individual variables						
T_{max} summer	+0.560	0.008				
Sunshine summer	+0.570	0.006	+0.462	0.030		
Rain days summer	-0.601	0.003	+0.425	0.049		
Sunshine autumn			+0.498	0.018	+0.581	0.005
Sunshine winter					+0.536	0.010

Abbreviations: EUB, effectively unbanded; FB, fully banded. Only correlations with $P < 0.05$ are shown. Weather variables were average values over the 2 years preceding collection.

snails (Figure 6). The mean annual intensity of selection was much lower for apex colour (0.017 ± 0.008) than for shell banding (0.122 ± 0.026), and was intermediate for apex colour in FB (0.051 ± 0.019).

The principal-components analysis of weather variables for each season produced clearly interpretable summary measures (Table 1). In summer and spring, the major component represented hot, sunny, dry conditions, whereas in autumn and winter, temperature was largely disconnected from the main component, which represented cloudy, wet conditions. Higher frequencies of EUB in the Acacia habitat over the 22-year period were associated with hotter, drier summers, as represented by summer PC1, and the individual variables of temperature, amount of sunshine and days without rain (Table 2). EUB was not correlated significantly with any weather variables in other seasons. The proportion of Pale within FB showed a very different pattern, with no correlations with summer conditions, but a positive correlation with sunnier, drier winters, as represented by winter PC1. Tests of association with individual variables gave a consistent pattern, in which only the amounts of sunshine in both autumn and winter were significant predictors of Pale within FB. The overall frequency of Pale, regardless of banding morph, showed an intermediate pattern, with no correlations with the principal components but positive correlations with hours of sunshine in summer and autumn (Table 2).

DISCUSSION

Interaction of apex colour and shell banding

This study shows that both apex colour and shell banding in *T. pisana* contribute to local adaptation, but with complicating effects of linkage, epistasis and contrasting details of selection. The effect of apex genotype on the intensity of shell banding means that the two traits are not simply different components of the appearance of the shell. The phenotypic disequilibrium also means that variation of apex colour cannot be understood on its own, but must be considered along with shell banding.

The phenotypic disequilibrium at Bold Park is in the same direction as found in three samples from Greece and Spain by Cowie (1984), who suggested that the disequilibrium is likely to be adaptive, but did not identify a mechanism. Linkage disequilibrium can also arise from founder effects, and it breaks down slowly with linked genes, so adaptive disequilibrium cannot be assumed. The parallel association of apex colour and banding with habitat at Bold Park is due partly to the phenotypic disequilibrium, in which Pale hitchhikes with EUB. Critically, however, Pale within FB shows the same association with habitat as EUB, indicating that these are independent components of a complex local adaptation. A caveat here is that the spatial pattern for apex colour is known only at this site, albeit with replication over two decades, and geographic replication will be necessary to confirm its generality. Nevertheless, the association with habitat is in the predicted direction, and the parallel association with banding is in the same direction as the phenotypic disequilibrium, which increases confidence that the association of Pale with EUB is adaptive, similar to the extensive evidence for adaptive disequilibrium between shell colour and banding in *C. nemoralis* (Cook, 2005). The likely disadvantage of EUB Dark in *T. pisana* is clear in terms of climatic selection, in which it is unlikely that dark shells would be favoured in small juveniles but pale shells in sub-adults and adults. In terms of camouflage, the EUB Dark phenotype is also likely to be a poor combination, as a distinctly dark apex on an otherwise white shell is conspicuous. Whatever the exact mechanism is, the rarity of the EUB Dark combination contrasts with the highly flexible combinations of colour and banding in the supergene of *C. nemoralis* (Cook, 2005), but parallels similar rarities in other land snails (for example, Murray and Clarke, 1976a,b; Asami and Asami, 2008) and mimetic butterflies (for example, Clarke and Sheppard, 1969; Joron *et al.*, 2006).

By contrast, the other three morphs have potential advantages. The epistasis means apex colour and banding are not phenotypically independent traits, and that FB Pale is an intermediate phenotype. The 'coupling' combinations, EUB Pale and FB Dark, are the extreme phenotypes in terms of intensity of pigmentation, and these show the extreme frequencies in the harsher Open habitat. Although micro-climatic selection is the most likely explanation for the increase in the frequency of pale shells in exposed habitats, the persistence of the polymorphism and the reversals in the directions of changes from year to year indicate that there must be another selective force (Johnson, 2011a). The obvious candidate is visual selection, which is likely to favour the banded snails in most habitats (Heller, 1981; Heller and Gadot, 1984). Whatever the particular selective mechanisms are, however, the intermediate FB Pale shows a more modest contrast between the habitats, consistent with a greater balance between the opposing selective forces.

Intensity of natural selection

Although apex colour and shell banding show parallel adaptation to habitat, banding has the greater effect on fitness. It is more strongly

associated with habitat, and it varies more between years, related to changes in the harshness of the summer. This has two effects on apex colour. First, the phenotypic disequilibrium leads to a hitchhiking effect on apex colour, whereby Pale increases directly with increases in EUB, although the contrasting temporal changes of the two traits indicate that this constraint is far from complete. Second, the phenotypic disequilibrium reduces the net selection on apex colour, because pale apices are found in both EUB and FB shells, which necessarily vary in opposite directions. The result is a constraint on apex colour, leading to a smaller difference between habitat and less temporal variation.

The estimates of annual selection coefficients within the Acacia habitat quantify this contrast and add to the scarce data on the intensity and variability of natural selection in polymorphic invertebrates. Both apex colour and shell banding fit the general picture that, in established populations in relatively unperturbed environments, the intensity of selection is generally low, but that it is sometimes substantial (Endler, 1986; Siepielski *et al.*, 2009). Even though the mean annual selection on apex colour was only 1.7%, compared with 12.2% for shell banding, it was as high as 15.3% (compared with 33.4% for EUB). The much lower selection on apex colour than on shell banding is at least partly due to the phenotypic disequilibrium. Selection on Pale within FB averaged 5.5%, and was high as 26.0%. Even the mean of 5.5% is greater than the average of 2% found in several long-term studies of shell morphs in the more widely studied *C. nemoralis*, although this may be due partly to the lack of annual sampling in those studies (see Johnson, 2011a). Thus, while apex colour faces less selection than shell banding, it is subject to sufficient selection to counter the effects of random changes in frequencies in all but very small populations, and to provide substantial evolutionary independence from shell banding.

The temporal patterns indicate that detailed mechanisms of selection differ for apex colour and shell banding, related to the stage of the life cycle. Summer is the harshest season for Australian populations of *T. pisana* (Baker, 2008; Johnson, 2011b), and it is not surprising that the frequency of banding morphs varies with summer conditions but not with winter conditions (Johnson, 2011a). By contrast, apex colour within the banded snails varies with winter sunshine and rainfall, which reflects the different stages of the life cycle affected by the two traits. Apex colour has its direct, strongest effect on the small juvenile snails, which are present in late autumn and winter, and the direct effects of selection on apex colour vary with those seasonal conditions. Although the mechanism of selection favouring EUB shells is almost certainly resistance to thermal stress (Heller, 1981; Hazel and Johnson, 1990; Johnson, 2011a), it is not as clear what favours pale apices in the banded population in sunnier, drier winters (or dark apices in cloudier, wetter winters). Heat stress is very unlikely, and the direction of the association argues against it. Darker shells absorb more heat, possibly giving dark apices an advantage in cloudier winter conditions, analogous to frost hollows favouring darker shells in *C. nemoralis* and *Cepaea hortensis* (Bantock, 1980; Cameron and Pokryszko, 2008). The winter temperatures in Perth, however, are moderate (average minimum of 7.7 °C in July, the coldest month, without frost in most years), so it is uncertain how important this is. Another conceivable mechanism is that the darker shells are more cryptic in darker conditions, so that temporal variation within the Acacia habitat is an indirect effect of climate, affecting selective predation. These are reasonable speculations, but experimental study is needed to determine the mechanisms of selection on the juvenile stage. Regardless of those details, both the parallel changes and the contrasting details of variation of banding and apex colour

illustrate the complexity of local adaptation. Such complexity is likely in adaptation to significant environmental contrasts, and this example in *T. pisana* shows both the constraints that phenotypic disequilibrium can place on the evolution of individual traits and the concerted responses of traits, each with their own specific adaptive mechanisms.

DATA ARCHIVING

Data have been deposited at Dryad: doi:10.5061/dryad.q318s.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ACKNOWLEDGEMENTS

The annual collections were performed as a project by my undergraduate class in genetics and evolution, and I thank the many students who contributed, as well as the graduate students who helped with supervision. Drs Robert Black, Wade Hazel and Jason Kennington also helped in various ways.

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Supplementary Information accompanies the paper on Heredity website (<http://www.nature.com/hdy>)