

Evolutionary change in *Cepaea nemoralis* shell colour over 43 years

MAŁGORZATA OŹGO* and MENNO SCHILTHUIZEN†‡

*Institute of Biology, Pomeranian University, ul. Arciszewskiego 22, 76-200 Słupsk, Poland, †Netherlands Centre for Biodiversity Naturalis, PO Box 9517, 2300 RA Leiden, The Netherlands, ‡Centre for Ecological and Evolutionary Studies, University of Groningen, PO Box 11103, 9700 CC Groningen

Abstract

We compared shell colour forms in the land snail *Cepaea nemoralis* at 16 sites in a 7 × 8 km section of the Province of Groningen, the Netherlands, between 1967 and 2010. To do so, we used stored samples in a natural history collection and resampled the exact collection localities. We found that almost all populations had experienced considerable evolutionary change in various phenotypes, possibly due to population bottlenecks and habitat change after repeated land consolidation schemes in the area. More importantly, we found a consistent increase in yellow effectively unbanded snails at the expense of brown snails. This is one of the expected adaptations to climate change (this area of the Netherlands has warmed by 1.5–2.0 °C over the time period spanned by the two sampling years), and the first clear demonstration of this in *C. nemoralis*.

Keywords: climate change, habitat change, Land snail, microevolution, natural history collections, polymorphism

Received 10 May 2011 and accepted 19 June 2011

Introduction

It is increasingly realized that natural history collections have the potential to provide unique data for investigating biogeographical and ecological trends over long time periods and thus play an important role in monitoring anthropogenic biodiversity change (Shaffer *et al.*, 1998; Godfray, 2002; Smith *et al.*, 2003). Less appreciated is the fact that they also may reveal *evolutionary* trends in traits that can be measured in preserved specimens. Species with short generation times, such as many invertebrates, may display rapid evolutionary responses to changing selection pressures (Parmesan, 2006; Harmon *et al.*, 2009). As many contemporary changes in selection are directly or indirectly human-induced, charting evolutionary trajectories of species in museum collections may provide means for monitoring and forecasting the evolutionary impacts of human actions. Carroll *et al.* (2005), for example, showed that proboscis length in preserved specimens of the bug *Leptocoris tagalicus* began to increase in specimens collected after 1965 – corresponding with the introduction of an invasive, large-seeded host plant. However, the heritability and genetic basis for many morphological traits are unknown. This makes it harder to interpret which part of the morphological change is due to evolutionary change (Wandeler *et al.*, 2007).

Colour polymorphisms are an exception: many colour polymorphisms have a simple genetic basis, allowing Mendelian genotypes to be assessed unambiguously and allele frequency changes charted (Majerus, 1998; Hoekstra, 2006; Galeotti *et al.*, 2009; Karell *et al.*, 2011).

The species with probably the best-studied genetically based colour polymorphism is the snail, *Cepaea nemoralis* (L.). This common and widespread European helioid land snail (introduced into North America) shows a complex range of colour variants that are determined by allelic variation in genes for shell ground colour (which ranges from pale yellow to deep brown), number of dark brown, spiral bands (zero to five) and band fusion (the merging of adjacent bands). Classical genetic studies have elucidated the inheritance and dominance relationships of all of the major genes and their alleles (Murray, 1975). Shell colour and banding pattern in *Cepaea* jointly affect the snails' thermoregulation, with lighter shells at an advantage under hot and exposed conditions (Heath, 1975; Steigen, 1979), presumably the cause for the latitudinal cline in shell colour found across Europe (Jones *et al.*, 1977; Silvertown *et al.*, 2011). On a smaller spatial scale, shell colour patterns also reveal the action of strong natural selection. Cain & Sheppard (1954) found that populations tended to be yellow and banded in open habitats, and darker (pink or brown) and effectively unbanded (shells with at least the top two bands missing) in shaded habitats. Based on data from shells broken by birds, they attributed this to visual predation by the

Correspondence: Menno Schilthuisen, tel. +31 71 5687769, fax +31 71 5687666, e-mail: menno.schilthuisen@ncbnaturalis.nl

song thrush (*Turdus philomelos* Brehm). These small-scale habitat associations have since been confirmed repeatedly (Jones *et al.*, 1977; Cook, 2008; Özgo, 2008; Silvertown *et al.*, 2011) although studies of bird predation as the chief selective agent have obtained mixed results (Cook, 2008). [It should be mentioned that much of the above also applies to *C. nemoralis*'s sister species *C. hortensis*, which is generally rarer and less widespread (Cook, 1998)].

C. nemoralis has a generation time of 2–3 years, a modal neighbourhood size of 6000 and rates of dispersal in the order of 5–10 m per year (Cook, 1998). High selection coefficients of up to 0.5 have been measured in *Cepaea* populations under directional or disruptive selection on colour morph alleles in the field (Cameron, 1992; Cameron & Pokryszko, 2008; Özgo & Kinnison, 2008). Given such potentially strong response to environmental selection and the sessile nature of the species, we may expect measurable human-induced changes in habitat and climate to leave their mark in the genetic make-up of *C. nemoralis* over periods of tens of snail generations. In this study, we aimed to investigate such changes by resampling sites in a small area in the Netherlands, where a set of museum samples was collected in the 1960s.

Methods

We checked the dry shell collection of the National Museum of Natural History Naturalis in Leiden, the Netherlands, for sets of *C. nemoralis* samples that were suitable as a basis for resampling. This meant that samples needed to meet the following criteria: (i) collected in a relatively small geographical area (100 × 100 m or less), to limit confounding phylogeographical variation; (ii) labels that allowed unambiguous location of the collection site with an accuracy of 100 m or better; (iii) collection year known; (iv) good condition (unfaded shells that could be scored for colour unambiguously). Using these criteria, we found a set of 73 samples (ranging in size from six to 154 individuals, in total 3180) collected between 4 and 25 July 1967 within an 8 × 7 km area directly northwest of the town of Winsum, Groningen province, the Netherlands (53° 20'N; 6°31'E). All were labelled with the three-digit 'Amersfoort' topographical coordinate system, which, using as a reference the same maps that probably would have been used in 1967 (i.e. sheets 7A and B, published 1961, and 3C and D, published 1962, of the 1 : 25 000 Topographic Service maps), allowed all sample sites to be identified with 10 m precision. In addition, many of the labels included sketches or notes on the habitat or details (e.g. 'trees north side of the road' or 'in dry ditch west of the road') that allowed even more exact replication of the collection conditions. The samples were collected by Dr. Henrik Wolda and co-workers as part of a long-term (1958–1971) research project on *Cepaea* at the University of Groningen (e.g. Wolda, 1969a, b; Wolda *et al.*, 1971). As far as we are aware, details of this set of samples have not been

published. All Wolda's samples were given additional permanent 'Özgo & Schilthuizen' labels (for this particular set, no. 20–66, 68–83 and 85–94).

We then scored shell colour phenotypes for all shells. The genetic basis of shell colour polymorphism in *C. nemoralis* is as follows (Murray, 1975). Shell ground colour is genetically controlled by a single locus, *C*, with multiple alleles coding for a range of colours from pale yellow via pink, to deep brown (in increasing dominance). Superimposed on the ground colour, the shell normally carries up to five spiral dark brown bands, which may or may not be fused. The *B* locus, linked with *C*, controls banding, with one dominant allele (for unbanded) suppressing a recessive allele (for banding). The dominant allele at the *T* locus (unlinked with *B* and *C*) acts epistatically to the *B* locus by suppressing bands 1 and 2, leaving only the three bands on the bottom of the shell. Similarly, the dominant allele at the *U* locus changes all banded morphs to mid-banded (removing bands 1, 2, 4 and 5). Shells with at least the top two bands missing appear unbanded in most views and are referred to as 'effectively unbanded'. The genetic control of fusion of adjacent bands, finally, is polygenic and not yet resolved. Shell phenotypes are normally scored with a coding system consisting of one letter (Y, P or B for the major colour morphs yellow, pink and brown), followed, to indicate banding, by a series of five digits (1–5), each replaced by a zero if the respective band is missing. Fusions of bands are indicated with parentheses around the band digits that are joined. The most common effectively unbanded yellow morphs, for example, have the phenotypes Y00000, Y00300 and Y00345, of which the latter may be Y00345, Y00(345), Y003(45) or Y00(34)5. We applied this phenotype scoring system to all 3180 shells of the '1967' samples.

For resampling, we chose the largest '1967' samples (i.e. comprising at least 30 shells). Some samples came from the same 10 × 10 m square or from adjoining squares, and were pooled together. In the field, we collected samples from 16 of those localities [listed in Table 1, S1 (Supporting information)]. For ease of reference, we have given these 16 sites topographic names (see Table 1). The exact locations were determined by comparison of the Amersfoort coordinates, the 1961/1962 1 : 25,000 maps and satellite photographs in Google Earth (date taken: 1 January 2005). Samples were taken by the authors on 6, 7 and 10 September, 2010. We collected all scoreable (i.e. adult and subadult) live snails as well as scoreable empty shells, either broken or unbroken, and kept these three groups separate. If there were two distinct habitats present at the site, we also kept those samples separate. Our '2010' samples ranged in size from 47 to 368 individuals per site, with a total of 2722. Phenotypes were scored, either in the field or in the lab, and the snails were then returned to the sites. Latitude and longitude coordinates were taken with a hand-held GPS, and the habitat was photographed. All phenotype data ('1967' and '2010' samples) were submitted to the Evolution Megalab database (www.evolutionmegalab.org), an online *Cepaea* colour polymorphism monitoring project.

To test for changes in phenotype frequencies, we compared frequencies per site. To do so, we first tested (using χ^2) whether significant phenotype frequency differences existed

Table 1 Sample sizes (N) and major phenotype frequencies at the 16 resampled sites, in 1967 and 2010 (Y = yellow; YeU = yellow; P = pink; B = brown; YeU = yellow, effectively unbanded, Y00XXX (as proportion of total); M = mid-banded, 00345 (as proportion of multiple-banded, i.e. 12345 + 00345 + all other multiple-banded morphs); Fusion = proportion of five-banded snails that belonged to the categories (12345), (123)(45), and (12)3(45). Statistical significance for each within locality comparison is given (χ^2 -test), as well as habitat change, as derived from observations in the field (2010) compared with aerial photographs (1960s)

Site name	Coordinates	year	N	Y	P	B	YeU	M	T	Fusion	Habitat (2010); change since 1967
Mensingeweer	53°21.345'N, 6°27.990'E	1967	695	0.263	0.506	0.230	0.069	0.288	0.028	0.664	Road-verge, tall
		2010	47	0.255 ^{ns}	0.638 ^{ns}	0.106*	0.085 ^{ns}	0.194 ^{ns}	0.034 ^{ns}	0.462 ^{ns}	herbs scattered trees, unchanged
Eenrum	53°21.404'N, 6°27.913'E	1967	89	0.213	0.539	0.247	0.045	0.433	0.053	0.419	Forest, become more
		2010	329	0.435 [†]	0.447 ^{ns}	0.119 [†]	0.249 [†]	0.449 ^{ns}	0.212*	0.582 ^{ns}	shaded
Ernstheem	53°21.776'N, 6°28.409'E	1967	34	0.412	0.588	0.000	0.118	0.138	0.200	0.667	Road-verge, tall herbs,
		2010	126	0.413 ^{ns}	0.508 ^{ns}	0.079 ^{ns}	0.103 ^{ns}	0.018 [†]	0.207 ^{ns}	0.435 ^{ns}	unchanged
Lutke-Saaxum-Bridge	53°22.104'N, 6°29.321'E	1967	154	0.091	0.539	0.370	0.000	0.272	0.030	0.349	Road-verge, hedgerow,
		2010	155	0.161 ^{ns}	0.465 ^{ns}	0.374 ^{ns}	0.026*	0.163 ^{ns}	0.078 ^{ns}	0.274 ^{ns}	unchanged
Lutke-Saaxum-Farm	53°22.068'N, 6°29.372'E	1967	56	0.196	0.393	0.411	0.036	0.242	0.000	0.565	Semi-open, become
		2010	205	0.259 ^{ns}	0.463 ^{ns}	0.278 ^{ns}	0.078 ^{ns}	0.232 ^{ns}	0.092 ^{ns}	0.213 [†]	more shaded
Baflo-West	53°21.969'N, 6°29.722'E	1967	57	0.123	0.807	0.070	0.088	0.468	0.560	0.636	Road-verge, semi-open,
		2010	220	0.282*	0.645*	0.073 ^{ns}	0.159 ^{ns}	0.371 ^{ns}	0.309*	0.460 ^{ns}	become more shaded
Baflo-East	53°21.943'N, 6°29.916'E	1967	120	0.058	0.817	0.125	0.033	0.260	0.130	0.724	Road-verge, hedgerow,
		2010	243	0.078 ^{ns}	0.749 ^{ns}	0.173 ^{ns}	0.012 ^{ns}	0.099 [†]	0.135 ^{ns}	0.550 ^{ns}	unchanged
Lutke-Saaxum-West	53°21.939'N, 6°29.178'E	1967	31	0.129	0.871	0.000	0.065	0.167	0.000	0.200	Road-verge, hedgerow,
		2010	165	0.303*	0.648*	0.048 ^{ns}	0.091 ^{ns}	0.291 ^{ns}	0.072 ^{ns}	0.219 ^{ns}	become more open
Matteneesse	53°21.432'N, 6°28.139'E	1967	149	0.275	0.430	0.295	0.114	0.232	0.110	0.549	Forest, unchanged
		2010	114	0.404*	0.491 ^{ns}	0.105 [†]	0.228*	0.519 [†]	0.000*	0.688 ^{ns}	
Roode Haan	53°19.811'N, 6°25.536'E	1967	147	0.367	0.626	0.007	0.088	0.219	0.000	0.400	Semi-open, become
		2010	115	0.383 ^{ns}	0.470*	0.148 [†]	0.165 ^{ns}	0.385*	0.050 ^{ns}	0.345 ^{ns}	more shaded
Winsum	53°20.087'N, 6°30.179'E	1967	55	0.291	0.673	0.036	0.145	0.256	0.188	0.105	Semi-open, become
		2010	92	0.239 ^{ns}	0.652 ^{ns}	0.109 ^{ns}	0.087 ^{ns}	0.254 ^{ns}	0.136 ^{ns}	0.375*	more shaded
Oude Tocht	53°22.167'N, 6°29.170'E	1967	42	0.595	0.190	0.214	0.024	0.061	0.097	0.704	Road-verge, hedgerow,
		2010	65	0.323 [†]	0.492 [†]	0.185 ^{ns}	0.046 ^{ns}	0.100 ^{ns}	0.028 ^{ns}	0.464 ^{ns}	unchanged
Handerweg-Zuid	53°22.358'N, 6°28.999'E	1967	51	0.098	0.725	0.176	0.000	0.000	0.049	0.538	Road-verge, hedgerow,
		2010	148	0.277 [†]	0.615 ^{ns}	0.108 ^{ns}	0.007 ^{ns}	0.078 ^{ns}	0.000*	0.748*	unchanged
Handerweg-Noord	53°22.488'N, 6°28.918'E	1967	82	0.427	0.049	0.524	0.037	0.051	0.000	0.724	Road-verge, hedgerow,
		2010	121	0.537 ^{ns}	0.190 [†]	0.273 [†]	0.174 [†]	0.271 [†]	0.000 ^{ns}	0.491*	unchanged
Houkumhuis	53°22.583'N, 6°30.514'E	1967	41	0.707	0.293	0.000	0.049	0.024	0.025	0.026	Semi-open, become
		2010	149	0.497*	0.430 ^{ns}	0.074 ^{ns}	0.181*	0.159*	0.264 [†]	0.213 [†]	more shaded

Table 1 (continued)

Site name	Coordinates	year	N	Y	P	B	YeU	M	T	Fusion	Habitat (2010); change since 1967
Houkumhuis-North	53°22.816'N, 6°30.427'E	1967 2010	79 50	0.557 0.260 [†]	0.443 0.640*	0.000 0.100 [†]	0.203 0.120 ^{ns}	0.359 0.344 ^{ns}	0.000 0.000 ^{ns}	0.000 0.000 ^{ns}	Road-verge; tall herbs scattered trees, unchanged
Overall (Mann-Whitney test)	—	—	—	ns	ns	ns	0.083	ns	ns	ns	—
Overall (Wilcoxon test)	—	—	—	ns	ns	ns	0.034	ns	ns	ns	—

Statistical significance:

ns: $P > 0.05$;

* $P < 0.05$;

† $P < 0.01$;

* $P < 0.001$ for each within locality comparison is given (χ^2 -test), as well as habitat change, as derived from observations in the field (2010) compared with aerial photographs (1960s).

among the various groups within sites (habitat, live, empty and broken, empty and unbroken), and pooled the data only when such differences did not exist. Then, we compared phenotype frequencies between the '1967' and '2010' samples. Per site, significance was determined with χ^2 -tests. To test for trends across all 16 sites, we applied Mann-Whitney and Wilcoxon paired-sample tests to the phenotype proportions. Finally, we assessed habitat changes between 1967 and 2010 by examining a set of aerial black-and-white photographs taken in 1968 from an altitude of 4 km and archived at the 'Kadaster' in Zwolle (Sheet 7, strips I and II, exposures 235–237 and 280–282) and comparing these with our field notes. Long-term temperature data (monthly minimum, maximum and mean) for the nearby weather station of Eelde (25 km S of Eenrum) were obtained from the Royal Netherlands Meteorological Institute.

Results

Figure 1 shows a map of the area with all sample sites indicated, and both the full '1967' set and the 16 sites that were resampled for the 1967–2010 comparison. Full phenotype, locality and habitat data for all '1967' and '2010' samples are given in Tables S1–S2; Table S1 also contains information on which sets of individuals were pooled for comparisons. Table 1 gives the phenotype frequency changes in all 16 sites between 1967 and 2010, as well as further information on site and on habitat change.

At almost all of the sites, one or more significant changes in some phenotype frequency occurred. Of those, the one that shows a more or less consistent change in the same direction (12 of 16 sites showed increase) across the samples is YeU, which increased (marginally) significantly. At the same time, habitat tended to become more shaded over time: of 16 sites, six became more shaded between 1967 and 2010 (see Fig. 2), which was mostly due to conversion of arable fields or pasture to woodland.

Finally, the temperature data for the Eelde weather station show increases of ca. 1.5 °C over the period 1960–2010 (especially the last two decades), for minimum, mean and maximum monthly temperatures (Fig. 3). Mean temperatures for the 2 years preceding our sample years (i.e. July 1965 through June 1967 and July 2008 through June 2010) are 8.6 and 9.2 °C respectively.

Discussion

Since the time that the '1967' samples were taken, environmental conditions of which impacts on shell phenotype fitness are known have changed in this small section of the province of Groningen. These concern site-specific changes as well as large-scale ones.



Fig. 1 The study area north-west of Winsum, Groningen Prov., The Netherlands. Symbols indicate the locations of all 73 samples (some sites were very close to others and are not shown individually). Closed symbols indicate the sites that were resampled in 2010.

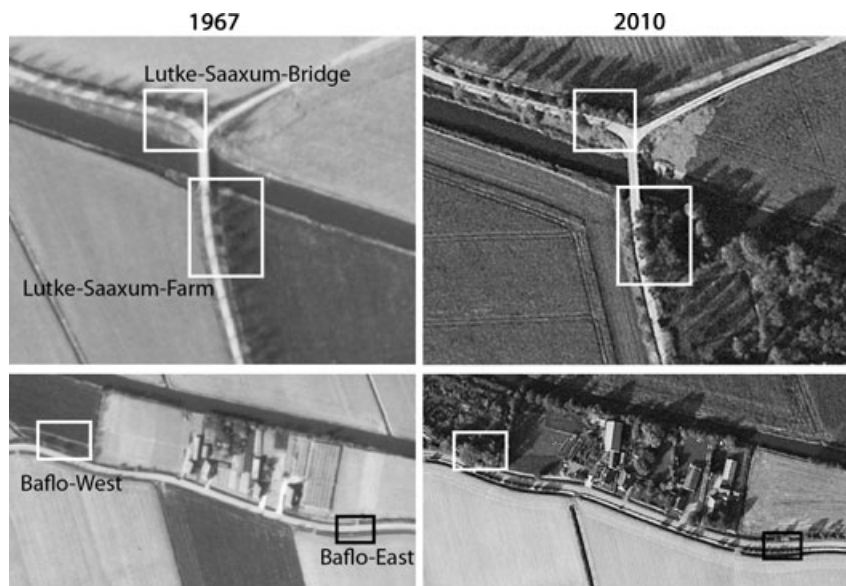


Fig. 2 Examples of habitat change. Sites Lutke-Saaxum-Bridge and Baflo-East are considered unchanged, whereas Lutke-Saaxum-Farm and Baflo-West have become more shaded due to conversion of arable fields or pasture to woodland. (The photos are meant to represent the 1967 and 2010 situations, but were actually taken in 1968 and 2005; also note that the season in which the photos were taken differs).

Two land consolidation projects took place (in the 1960s and from 1985 onwards; Gemeente De Marne 2011), which led to changes in infrastructure as well as in land use, with frequent conversion of arable land and pasture to recreational forest. In addition, and more generally, the province of Groningen shows the same trends in land use as the rest of the Netherlands (and, indeed, Western Europe): increase of forest cover

and built-up areas and decrease of agricultural land use [forest cover in Groningen has increased from 1135 ha in the mid-1960s to 6765 in 2005 (CBS, PBL, Wageningen UR 2006)]. The effects of this are immediately visible in the study sites: at six of the 16 sites, the habitat became more shaded, mostly because trees now grow where there was agricultural land in the past. In only one site did the habitat become less

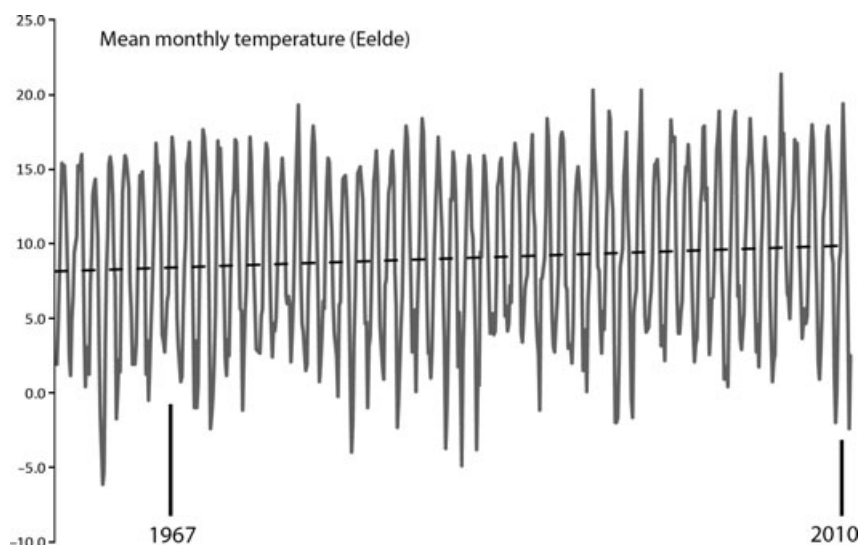


Fig. 3 Mean monthly temperatures (in °C) from January 1960 to February 2011, with trend-line fitted, for weather station Eelde (source: KNMI, Royal Netherlands Meteorological Institute).

shaded. Changes in the song thrush population are probably negligible: song thrush density in this part of Groningen is among the lowest in the Netherlands, and major changes in density have not been recorded since 1970 (van Diermen 2002). As for large-scale environmental changes, the chief one is climate change. From 1960 until 2010, temperatures increased by 1.5–2.0 °C in this part of the Netherlands, as they did elsewhere in the country (Royal Netherlands Meteorological Institute 2011).

On the basis of what we know of the adaptive responses of *Cepaea* shell colouration to habitat and temperature, we would expect that (i) populations at sites that have become more shaded would have become darker, whereas (ii) overall, the temperature increase would have resulted in shells becoming lighter. Our first prediction is not borne out. Table 1 shows that significant changes took place at almost all sites for one or more phenotypes. For example, at Eenrum, yellow went up from 0.21 to 0.44 ($P < 0.001$); at Ernstheem, mid-banded went down from 0.14 to 0.02 ($P < 0.01$); at Lutke-Saaxum-Farm, fusion decreased from 0.57 to 0.21 ($P < 0.001$); at Houkumhuis, three-banded increased from 0.03 to 0.26 ($P < 0.001$); and at Oude Tocht, pink went from 0.19 in 1967 to 0.49 in 2010 ($P < 0.01$). Sites like Mattensse, Handerweg-Noord and Houkumhuis even showed significant changes at almost all of the examined phenotype classes. However, for the most part, these changes are not consistent across sites: most phenotypes changed in a minority of the sites and in different directions, and do not appear to be related to any change in habitat type. Consequently, the Mann–Whitney and Wilcoxon tests for all

paired samples yield non-significant results for all phenotypes except yellow effectively unbanded (YeU; see below).

This suggests two things. First, considerable evolutionary change has taken place in almost all of the sites. Second, much of this change is small-scale and idiosyncratic, the result of genetic drift, extinction and recolonization or undetected and extremely local changes in environmental selection or a combination of these. Such idiosyncratic change is in line with the observation that genetic differentiation was high to begin with: e.g. Handerweg-Noord and Handerweg-Zuid, only a few 100 m apart and in the same habitat, showed very different phenotype frequencies in 1967 (e.g. in yellow, pink, and brown proportions), as they do today, and yet both show significant temporal changes in phenotype frequencies.

Local changes in the strength and direction of selection probably played a part. Of the 16 sites, 10 were roadside verges. Habitats at such sites are probably extremely unstable: hedges can provide shaded conditions, but are cut down periodically. At some sites, almost total removal of vegetation is occasionally carried out. These changes, although dramatic from the snail's point of view, cannot be inferred from aerial photographs, and thus remained undetected. Given that in a highly polymorphic species, such as *C. nemoralis*, various phenotypes can be selected for under similar selective conditions (Ozgo, 2011), the idiosyncratic responses observed in our study are not unexpected.

The changes in habitat we were able to detect were towards greater shadiness, which should have selected for darker snails. However, the one consistent change

in phenotype frequencies is towards lighter snails: an increase in YeU. In 12 sites, there is an increase, and in four sites a decrease; this is significant in paired-sample comparison. This increase in YeU confirms our second prediction, namely, due to climate change, snails would have become lighter in colour; in fact, snails have become lighter in spite of habitat change.

Our results are interesting in the light of other resampling studies of *C. nemoralis*. The conspicuous evolutionary dynamism of our sites contrasts markedly with those studied by Goodhart (1956), Wolda (1969a), Cain *et al.* (1990) and Arthur *et al.* (1993), who all emphasize phenotype stability over the periods spanned by sampling and resampling (which range from 6 to 64 years). Some studies detected increases in yellow at the expense of brown, but those changes were slight, and often inconsistent (Cowie & Jones, 1998; Cook *et al.*, 1999). Small and unexplained increases in mid-banded were also noted in some areas (Clarke & Murray, 1962a, b; Cook & Pettitt, 1998; Cameron, 2001); Silvertown *et al.* (2011) reported an increase in mid-banded over the past 50 years across the whole of Europe. Thus, our study is among the first to detect consistent evolutionary change attributable to global climatic warming in *C. nemoralis*. One reason might be that previous studies tended to focus on genotypes, such as yellow or unbanded, which are phenotypically mixed and may contain both light and dark shells: e.g. yellow unbanded (relatively light) and yellow five-banded (relatively dark). In fact, in our study, not a single genotype showed a consistent change over time; the detectable change was in a genetically mixed, but phenotypically more uniform light shell category (YeU). Possibly, the increase in mid-banded observed in other studies (see above) in fact indicates an increase in effectively unbanded.

The change detected in our study, although measurable and fairly consistent, is small in magnitude. This contrasts with high rates of evolutionary change observed in some studies on *Cepaea* snails (Cameron, 1992; Cameron & Pokryszko, 2008; OŹgo & Kinnison, 2008), but is consistent with the observation that strong directional selection is rarely sustained over prolonged time periods (Hoekstra *et al.*, 2001; Reznick & Ghalambor, 2001; Bell, 2010). Indeed, variations in temperature experienced from year-to-year by the snails are far greater than increases in the many-year average, and studies on the related snail species *C. hortensis* (Cameron & Pokryszko, 2008) and *Theba pisana* (Johnson, 2011a, b; Schilthuisen, 2011) have shown that the response to the temperature conditions immediately preceding the time of sampling have the greatest effect on phenotype frequencies, with relatively small net changes over longer time periods. In our case, tempera-

ture differences between the two 2-year periods preceding sampling in 1967 and 2010 were relatively small (the recent period was 0.6 °C warmer than the older one) compared with the long-term trend. It remains to be investigated whether year-to-year fluctuations or long-term trends are chiefly responsible for the shell colour evolution we found.

In conclusion, our results show a small increase in YeU over the majority of sites, which fits with the notion that climate warming has selected for lighter shells. In addition, we observe that populations at almost all our sites have experienced idiosyncratic evolutionary changes, which is probably the result of selection and/or bottlenecks induced by habitat instability, although the phenotype changes cannot be easily interpreted as a direct result of any observed habitat changes. Finally, we stress the relevance of storing material from any invertebrate sampling project in natural history collections. As this material has not been published before, and the laboratory records of the Wolda project cannot be located anymore (W. van Delden, pers. comm.), our study would not have been possible otherwise.

Acknowledgements

We thank the staff of the Collections department at NCB Naturalis (Jeroen Goud, Caroline Pepermans, and Kees van den Berg) for helping us to locate, for curating, and for making accessible to us the *Cepaea* samples. Liew Thor-Seng, Nicole Webster, and Ralph Boon von Ochssee assisted in the field. Henk Wolda (Seattle), Lucy Kurstjens-Oosterhoff (Nijmegen), Rinny Kooi (Leiden University), the librarians at the Centre for Ecological and Evolutionary Studies of Groningen University, and Professor Wilke van Delden were extremely helpful in providing first-hand information on collection localities, and in trying (but failing) to locate historical lab records. The Kadaster Zwolle assisted in finding aerial photographs from the 1960s, and the Royal Netherlands Meteorological Institute provided historical weather data for Eelde. Finally, the Evolution Megalab coordinators (Jonathan Silvertown and Mike Dodd at the Open University) helped store our data in the Megalab records. MO is grateful to Mirjam Houtlosser for hospitality in the Netherlands.

References

- Arthur W, Phillips D, Mitchell P (1993) Long-term stability of morph frequency and species distribution in a sand-dune colony of *Cepaea*. *Proceedings of the Royal Society of London B*, **251**, 159–163.
- Bell G (2010) Fluctuating selection: the perpetual renewal of adaptation in variable environments. *Philosophical Transactions of the Royal Society of London B*, **365**, 87–97.
- Cain AJ, Sheppard PM (1954) Natural selection in *Cepaea*. *Genetics*, **39**, 89–116.
- Cain AJ, Cook LM, Currey JD (1990) Population size and morph frequency in a long-term study of *Cepaea nemoralis*. *Proceedings of the Royal Society of London B*, **240**, 231–250.
- Cameron RAD (1992) Change and stability in *Cepaea* populations over 25 years: a case of climatic selection. *Proceedings of the Royal Society of London B*, **248**, 181–187.
- Cameron RAD (2001) *Cepaea nemoralis* in a hostile environment: continuity, colonizations and morph-frequencies over time. *Biological Journal of the Linnean Society*, **74**, 255–264.

- Cameron RAD, Pokryszko B (2008) Variation in *Cepaea* populations over 42 years: climate fluctuations destroy a topographical relationship of morph-frequencies. *Biological Journal of the Linnean Society*, **95**, 53–61.
- Carroll SP, Loye JE, Dingle H, Mathieson M, Famula TR, Zalucki MP (2005) And the beak shall inherit – evolution in response to invasion. *Ecology Letters*, **8**, 944–951.
- CBS, PBL, Wageningen UR (2006) Bosareaal Provincies (indicator 1159, versie 04). Available at: www.compendiumvoordeleefomgeving.nl (accessed 13 December 2006). CBS, Den Haag; Planbureau voor de Leefomgeving, Den Haag/Bilthoven and Wageningen UR, Wageningen.
- Clarke B, Murray J (1962a) Changes in gene frequency in *Cepaea nemoralis* (L.). *Heredity*, **17**, 445–465.
- Clarke B, Murray J (1962b) Changes in gene frequency in *Cepaea nemoralis* (L.); the estimation of selective values. *Heredity*, **17**, 467–476.
- Cook LM, Cowie RH, Jones JS (1999) Change in morph frequency in the snail *Cepaea nemoralis* on the Marlborough Downs. *Heredity*, **82**, 336–342.
- Cook LM (1998) A two-stage model for *Cepaea* polymorphism. *Philosophical Transactions of the Royal Society of London, Series B*, **353**, 1577–1593.
- Cook LM, Pettitt CWA (1998) Morph frequencies in the snail *Cepaea nemoralis*: changes with time and their interpretation. *Biological Journal of the Linnean Society*, **64**, 137–150.
- Cook LM (2008) Variation with habitat in *Cepaea nemoralis*: the Cain and Sheppard diagram. *Journal of Mollusc Studies*, **74**, 239–243.
- Cowie RH, Jones JS (1998) Gene frequency changes in *Cepaea* snails on the Marlborough Downs over twenty-five years. *Biological Journal of the Linnean Society*, **65**, 233–255.
- van Diermen J (2002) Zanglijster *Turdus philomenos*. In: *Atlas van de Nederlandse Broedvogels 1998–2000* (ed. SOVON Vogelonderzoek Nederland), pp. 362–363. Nationaal Natuurhistorisch Museum Naturalis, KNNV Uitgeverij & European Invertebrate Survey-Nederland, Leiden.
- Galeotti P, Rubolini D, Sacchi R, Fasola M (2009) Global changes and animal phenotypic responses: melanin-based plumage redness of scops owls increased with temperature and rainfall during the last century. *Biology Letters*, **5**, 532–534.
- Gemeente De Marne (2011) Website of the Council of De Marne. Available at: <http://www.demarne.nl> (accessed 9 April 2011).
- Godfray CM (2002) Challenges for taxonomy. *Nature*, **417**, 17–19.
- Goodhart CB (1956) Genetic stability in populations of the polymorphic snail, *Cepaea nemoralis* (L.). *Proceedings of the Linnean Society of London*, **167**, 50–67.
- Harmon JP, Moran NA, Ives AR (2009) Species response to environmental change: impacts of food web interactions and evolution. *Science*, **323**, 1347–1350.
- Heath DJ (1975) Color, sunlight and internal temperatures in the land snail *Cepaea nemoralis*. *Oecologia*, **19**, 29–38.
- Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hoang CE, Beerli P (2001) Strength and tempo of directional selection in the wild. *Proceedings of the National Academy of Sciences USA*, **98**, 9157–9160.
- Hoekstra HE (2006) Genetics, development and evolution of adaptive pigmentation in vertebrates. *Heredity*, **97**, 222–234.
- Johnson MS (2011a) Thirty-four years of climatic selection in the land snail *Theba pisana*. *Heredity*, **106**, 741–748.
- Johnson MS (2011b) Epistasis, phenotypic disequilibrium, and contrasting associations with climate in the land snail *Theba pisana*. *Heredity*, **106**, 741–748 (in press).
- Jones JS, Leith BH, Rawlings P (1977) Polymorphism in *Cepaea*: a problem with too many solutions? *Annual Review of Ecology and Systematics*, **8**, 109–143.
- Karell P, Ahola K, Karstinen T, Valkama J, Brommer JE (2011) Climate change drives microevolution in a wild bird. *Nature Communications*, **2**, 208.
- Majerus MEN (1998) *Melanism, Evolution in Action*. Oxford University Press, Oxford.
- Murray J (1975) The genetics of the Mollusca. In: *Handbook of Genetics*, Vol 1, (ed. King RC), pp. 3–31. Plenum Press, New York.
- Ozgo M (2008) Current problems in the research of *Cepaea* polymorphism. *Folia malacologia*, **16**, 55–60.
- Ozgo M (2011) Rapid evolution in unstable habitats: a success story of the polymorphic land snail *Cepaea nemoralis* (Gastropoda: Pulmonata). *Biological Journal of the Linnean Society*, **102**, 251–262.
- Ozgo M, Kinnison MT (2008) Contingency and determinism during convergent contemporary evolution in the polymorphic land snail, *Cepaea nemoralis*. *Evolutionary Ecology Research*, **10**, 721–733.
- Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology and Systematics*, **37**, 637–669.
- Reznick DN, Ghalambor CK (2001) The population ecology of contemporary adaptations: what do empirical studies reveal about the conditions that promote adaptive evolution. *Genetica*, **112–113**, 183–198.
- Royal Netherlands Meteorological Institute (2011) Climate Scenarios. Available at: http://www.knmi.nl/klimaatsscenario/waarnemingen/monitoring_temperature.php (accessed 9 April 2011).
- Schilthuizen M (2011) Scrutinising snail shells. *Heredity*, **106**, in press.
- Shaffer HB, Fisher RN, Davidson C (1998) The role of natural history collections in documenting species declines. *Trends in Ecology and Evolution*, **13**, 27–30.
- Silvertown J, Cook L, Cameron R *et al.* (2011) Citizen science reveals unexpected continental-scale evolutionary change in a model organism. *PLoS ONE*, **6**, e18927.
- Smith GF, Steenkamp Y, Klopper RR, Siebert SJ, Arnold TH (2003) The price of collecting life. *Nature*, **422**, 375–376.
- Steigen AL (1979) Temperature effects on energy metabolism in banded and unbanded morphs of the snail *Cepaea hortensis*. *Oecologia*, **41**, 163–174.
- Wandeler P, Hoek PEA, Keller LF (2007) Back to the future: museum specimens in population genetics. *Trends in Ecology and Evolution*, **22**, 634–642.
- Wolda H (1969a) Stability of a steep cline in morph frequencies of the snail *Cepaea nemoralis* (L.). *Journal of Animal Ecology*, **38**, 623–635.
- Wolda H (1969b) Distribution of morph frequencies in the snail, *Cepaea nemoralis* near Groningen. *Journal of Animal Ecology*, **38**, 305–327.
- Wolda H, Zweep A, Schuitema KA (1971) The role of food in the dynamics of populations of the landsnail *Cepaea nemoralis*. *Oecologia*, **7**, 361–381.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Composition of the samples included in paired-sample comparisons.

Table S2. Composition of the samples not included in the paired-sample comparisons.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.