ORIGINAL ARTICLE
Rapid, habitat-related evolution of land snail colour morphs on reclaimed land

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I made use of the known dates of reclamation (and of afforestations) in the IJsselmeerpolders in The Netherlands to assess evolutionary adaptation in *Cepaea nemoralis*. At 12 localities (three in each polder), I sampled a total of 4390 adult individuals in paired open and shaded habitats, on average 233 m apart, and scored these for genetic shell colour polymorphisms. The results show (highly) significant differentiation at most localities, although the genes involved differed per locality. Overall, though, populations in shaded habitats had evolved towards darker shells than those in adjacent open habitats, whereas a 'Cain & Sheppard' diagram (proportion yellow shells plotted against 'effectively unbanded' shells) failed to reveal a clear pattern. This might suggest that thermal selection is more important than visual selection in generating this pattern. Trait differentiation, regardless of whether they were plotted against polder age or habitat age, showed a linear increase of differentiation with time, corresponding to a mean rate of trait evolution of 15–31 kilodarwin. In conclusion, *C. nemoralis* is capable of rapid and considerable evolutionary differentiation over 1–25 snail generations, though equilibrium may be reached only at longer time scales.
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INTRODUCTION

In many animal and plant species, separate colour morphs exist within populations. Such colour polymorphisms have long been considered suitable trait systems to study contemporary evolution (Ford, 1945; Endler, 1986; Forsman *et al.*, 2008), for several reasons. First, discrete colour morphs can often be scored easily and unambiguously in large numbers of individuals. Second, pigmentation type and pattern often have high heritability and a simple genetic basis (Reillo and Wise, 1988; Stewart and Lees, 1988; Wittkopp *et al.*, 2009). Finally, colouration frequently responds strongly to natural and sexual selection (Gigord *et al.*, 2001; Bell, 2008; Cook *et al.*, 2012). Indeed, many studies demonstrating considerable contemporary evolution have made use of colour polymorphisms in organisms such as freshwater fish (Endler, 1980), intertidal and terrestrial snails (Hughes and Mather, 1986; Johnson, 2011), and owls (Karell *et al.*, 2011).

One of the best known and most intensively studied model systems for selection on colour polymorphism is the land snail *Cepaea*. In *Cepaea nemoralis* and *C. hortensis*, two common European species, very conspicuous, and heritable (Murray, 1975), variation exists in shell colour and colour pattern. Shell ground colour ranges from bright yellow to deep brown and superimposed on this a variable pattern of dark brown spiral bands may exist (Lang, 1904; Goodhart, 1987). Numerous studies have shown that natural selection acts strongly on many of these colour morphs under different circumstances (though *C. nemoralis* often differs from *C. hortensis* in the exact response; Clarke, 1960). Evidence exists for selection for habitat-dependent cryptic colouration by predation by the song thrush, *Turdus philomelos* (Cain and Sheppard, 1950, 1954; Lamotte, 1951; Carter, 1968). On the other hand, the effects of shell colour on the snails' thermoregulation (Heath, 1975) create a separate selection pressure, leading to summertime selection for shells with higher albedo in exposed habitats (Cain, 1968; Jones *et al.*, 1977) and lower latitudes (Jones *et al.*, 1977; Silvertown *et al.*, 2011), and wintertime selection for light-coloured shells in topographies that are prone to extreme cold events (Cameron, 1969). In addition, some effects of shell colour on behaviour have also been reported: Wolda (1965) and Jones (1982) found that different colour morphs have different tendencies to climb trees, and to rest in exposed sites. Finally, phylogeographic patterns in allele predominance (so-called 'area effects') exist that may not be related to any direct environmental selection (Cain and Currey, 1963; Davison and Clarke, 2000). Because of this mixture of evolutionary forces and the spatial scales at which they operate, it has often been difficult to untangle the precise mechanism for observed colour differentiation (Jones *et al.*, 1977; Cook, 1998).

Although direct and conclusive evidence for the type and strength of selection is available only in a few cases, many studies confirm that shaded and open habitats in the same area (where the larger-scale effects do not interfere) have significantly different morph frequencies. In general, shaded habitats tend to have lower frequencies of yellow and higher frequencies of effectively unbanded (Cain and Sheppard, 1950, 1954), although the latter effect does not appear everywhere (Lamotte, 1966; Cook, 2008; Silvertown *et al.*, 2011; Cameron and Cook, 2012b). Also, populations in shaded habitats often are reported to have higher frequencies of band fusion (Clarke, 1960; Richards and Murray, 1975; Ożgo, 2011).

This habitat-related selection in *Cepaea* is often quoted as an example of contemporary, though relatively slow (requiring at least

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100 generations; Cook, 1998) evolution. However, only in a few studies are the ages of the populations and/or the habitats known with some certainty, allowing evolutionary rates to be estimated. For example, Richards and Murray (1975) made use of a *C. nemoralis* population introduced into North America, and demonstrated that, in this population fixed for yellow shell ground colour, shaded habitat populations had obtained significantly higher banding and higher band fusion frequencies over a period of at least 43 years. Similarly, Ożgo and Bogucki (2011) performed a transplant experiment in Poland, showing that, over 11 years, frequencies of yellow and yellow effectively unbanded in *C. nemoralis* diverged slightly but significantly between open and shaded habitats. Ożgo and Kinnison (2008) detected strong shell colour divergence in three open/sheltered habitat pairs in southeastern Poland that had presumably been established 18–28 years previously. Ożgo (2011), finally, surveyed 12 newly-established open/shaded habitat pairs in Germany and Poland (presumably 60–80 years old) and concluded that most of these showed significantly higher frequencies of yellow and lower frequencies of band fusion in the open habitats.

These studies suggest that, with a generation time of around 3 years (Jones *et al.*, 1977; Cook, 1998), *Cepaea* populations can diverge under habitat-associated selection in time spans much less than 100 generations. However, it may be argued that larger series of time-controlled (natural) experiments with known dates of population establishment are needed for a proper assessment of evolutionary rates in *Cepaea*. Here, I adopt the Dutch IJsselmeerpolders to provide such a system. These large areas of reclaimed land (*polders*) around the former Zuiderzee estuary were drained in the mid-20th century and subsequently colonised by flora and fauna, including *C. nemoralis*. As the *C. nemoralis* populations inhabiting paired open/shaded habitats in these polders cannot be older than the polders themselves, and as the four major polders were drained with 11–15 intervening years, they allow evolutionary rates to be estimated with some accuracy.

MATERIALS AND METHODS
Genetics and scoring system for the *Cepaea* shell colour polymorphism
Murray (1975) summarises the genetics of the shell colour polymorphism in *C. nemoralis*. The locus *C* controls shell ground colour, with alleles that determine colours ranging from pale yellow to deep brown (in increasing dominance). The shell may also carry one to five dark brown spiral bands, which are sometimes fused with one another. Banding is controlled by the *B* locus (linked with *C*). The dominant allele (for unbanded) suppresses a recessive allele for banding. At the unlinked *T* locus the dominant allele suppresses bands 1 and 2, leaving only the three lowest bands. The dominant allele at the *U* locus removes bands 1, 2, 4 and 5, making shells mid-banded. All banding categories with the top two bands absent are termed 'effectively unbanded'. The polygenic genetic control of band fusion, finally, is not yet resolved. Shell phenotypes are conventionally coded with a letter (Y, P or B) for the ground colour (yellow, pink and brown), followed by five digits (1–5), each replaced by a zero for a missing band. Band fusions (determined a quarter of a whorl away from the aperture) is coded with parentheses around the band digits that are joined. As an example, the following morphs are all effectively unbanded pink: P00000, P00300, P00345, P00(345), P003(45) and P00(34)5.

Study areas and sampling
The IJsselmeerpolders comprise four large areas in the north-central part of The Netherlands (see Figure 1): Wieringermeer (WI; 193 km², drained in 1930), Noordoostpolder (NOP; 469 km², 1942), Oostelijk Flevoland (OF; 528 km², 1957) and Zuidelijk Flevoland (ZF; 430 km², 1968). Today, the areas consist mostly of agricultural land, but also large industrial and residential areas exist, with scattered wetland and woodlands. To select habitat

pairs, I used satellite images available in Google Earth and visited many suitable habitat pairs in the winter of 2010/2011, and, based on the availability of sufficient empty *Cepaea* shells, selected 12 pairs (three in each polder; see Figure 1 and Supplementary Table 1). Distances between the two habitats in a pair averaged 233 m (range: 35 to 650 m) and were thus less than the minimum of 1 km recommended by Cameron and Cook (2012a). Using the historical aerial photographs available at http://historische-luchtfoto.flevo-land.nl/, editions of topographic maps spanning much of the 20th century, and interviews with local foresters, I also estimated the year (in most cases ± 3 years) in which each shaded habitat had appeared. In the remainder of this paper, I shall use the term 'locality' for each of the 12 places where habitat pairs were sampled, the term 'site' for a single habitat plot, and 'sample' for the snails collected in a site.

The sites were sampled between 11 May and 23 September, 2011. At each site, characteristics of the vegetation were recorded, and adult *C. nemoralis* snails were collected in a systematic fashion within a plot of <5000 m². I aimed to obtain at least 150 individuals from each of the 24 sites. Colour morphs were scored in the field, after which the snails were released back into the site. No attempt was made to quantify bird predation. Data were submitted to the online *Cepaea* polymorphism database Evolution Megalab (www.evolutionmegalab.org). Bird data show that all sites fell within areas with high densities of the song thrush (van Diermen, 2002). The soil at all sites consists of young marine clay with a high density of mollusk shells, except for Voorsterbos, which is on a fluvioglacial deposit (Wolters-Noordhoff, 2001).

Analyses
For each sample, I calculated the proportions (for all snails) of yellow, pink and brown, and unbanded, as well as effectively unbanded. I also calculated the proportions of all band combinations (of which 00300, 00345 and 12345 were the most abundant) among the banded category, and a measure for band fusion by taking the proportion of 5-banded snails with at least two bands fused. Differences in morph frequencies within each habitat pair were tested with χ^2-tests. The euclidean distances generated from the morph frequency data were used to generate a phenogram for all samples using Ward-method in PAST 2.13 (Hammer *et al.*, 2001). The pattern in frequencies yellow and effectively unbanded was inspected via a 'Cain and Sheppard diagram' (Cook, 2008).

In addition, for each snail, a 'darkness score' was determined. For this, I applied the data in Heath (1975) on the thermal properties of different colour morphs as follows. Using yellow unbanded as the base line, the expected temperature increases of other morphs were calculated by adding 0.3 °C for pink, 0.6 °C for brown, 0.07 °C for each band and 0.03 °C for each band fusion. The resultant expected temperature increase was used as 'darkness score'. Differences in darkness between two sites of the same locality were tested for significance with Kruskall–Wallis one-way analysis of variance, and the overall significance was tested with Fisher's combined probability test.

Finally, for each habitat pair, I calculated a standardised measure for differentiation between the open and the shaded habitat for each of the major morph categories (that is, frequencies of P, B, YeU, banded, eU, 00300, 00345, 12345, fusion in 5-banded and fusion in all banded). I did this by taking the absolute value of $\log(P_O/P_S)$, in which P_O is the frequency of the morph concerned in the open habitat, and P_S in the shaded habitat. These differentiation measures were then correlated with polder age (see Figure 1) and estimated age of the shaded habitat (Table 1). Significance was assessed with an F-test. Under the assumption that the open habitat represented the original morph frequency and the shaded habitat represented the differentiated state, evolutionary rate was calculated (and expressed in kilodarwins; Haldane, 1949) as $(\ln P_S - \ln P_O)/t$ for all major morph categories (where *t* is the time period in 10^3 year).

RESULTS
In most sites, I obtained samples of around 150 individuals or more, except for Nagele Open and Shaded and Cirkelbos Open, where numbers were just under 100. Total number of snails collected was 4390. The cluster analysis (Figure 2) shows some phylogeographic patterns: for five localities (Knarbos, Wilgenreservaat, Nagele,
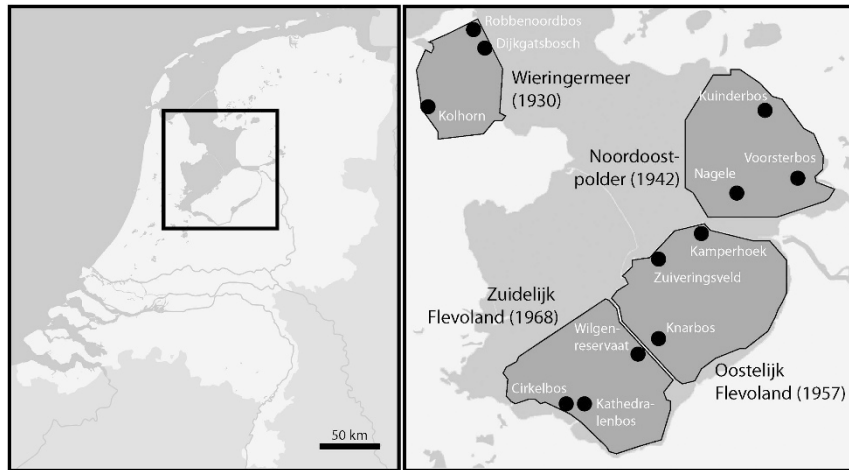
Figure 1 Map of The Netherlands (left) with the IJsselmeer (inset), the four land reclamation projects (and the years of draining) shown in dark grey. Black dots indicate the localities used in this study.

Table 1 Morph frequencies and ages of sites

Site name	Open/closed	N	Yellow	Pink	Brown	Yellow (eU)	00300	00345	12345	fusion
Kuinderbos	Shaded (46 year)	197	101 (0.51)	36 (0.18)	60 (0.30)	67 (0.34)	3 (0.05)	21 (0.34)	33 (0.53)	18 (0.55)
Kuinderbos	Open	182	134 (0.74)	36 (0.20)	12 (0.07)	113 (0.62)	21 (0.20)	53 (0.50)	21 (0.20)	1 (0.05)
			***	NS	***	***	**	*	***	***
Nagele	Shaded (56 year)	97	3 (0.03)	43 (0.44)	51 (0.53)	1 (0.01)	0 (0.00)	1 (0.03)	36 (0.90)	14 (0.39)
Nagele	Open	91	3 (0.03)	73 (0.80)	15 (0.16)	0 (0.00)	0 (0.00)	0 (0.00)	44 (1.00)	7 (0.16)
			NS	***	***	NS	NS	NS	NS	*
Voorsterbos	Shaded (7 year)	196	169 (0.86)	21 (0.11)	6 (0.03)	92 (0.47)	6 (0.04)	83 (0.49)	76 (0.44)	9 (0.12)
Voorsterbos	Open	175	111 (0.63)	55 (0.31)	9 (0.05)	87 (0.50)	45 (0.37)	44 (0.36)	25 (0.21)	3 (0.12)
			***	***	NS	NS	***	*	***	NS
Knarbos	Shaded (36 year)	179	102 (0.57)	30 (0.17)	47 (0.26)	17 (0.09)	17 (0.16)	2 (0.02)	89 (0.82)	43 (0.48)
Knarbos	Open	219	84 (0.38)	48 (0.22)	87 (0.40)	3 (0.01)	2 (0.02)	1 (0.01)	92 (0.96)	39 (0.42)
			***	NS	**	***	***	NS	**	NS
Zuiveringsveld	Shaded (46 year)	153	52 (0.34)	72 (0.47)	29 (0.19)	46 (0.30)	21 (0.31)	24 (0.35)	17 (0.25)	4 (0.24)
Zuiveringsveld	Open	228	78 (0.34)	95 (0.42)	55 (0.24)	69 (0.30)	30 (0.27)	55 (0.50)	18 (0.16)	3 (0.17)
			NS	NS	NS	NS	NS	NS	NS	NS
Kamperhoek	Shaded (36 year)	223	136 (0.61)	46 (0.21)	41 (0.18)	106 (0.48)	47 (0.28)	77 (0.46)	40 (0.24)	25 (0.63)
Kamperhoek	Open	185	118 (0.64)	14 (0.08)	53 (0.29)	105 (0.57)	34 (0.31)	63 (0.57)	13 (0.12)	2 (0.15)
			NS	***	*	NS	NS	NS	*	**
Dijkgatsbosch	Shaded (57 year)	151	98 (0.65)	31 (0.21)	22 (0.15)	71 (0.47)	5 (0.06)	43 (0.48)	35 (0.39)	13 (0.37)
Dijkgatsbosch	Open	155	52 (0.34)	85 (0.55)	18 (0.12)	28 (0.18)	17 (0.15)	46 (0.41)	48 (0.43)	3 (0.06)
			***	***	NS	***	*	NS	NS	***
Kolhorn	Shaded (69 year)	239	185 (0.77)	3 (0.01)	51 (0.21)	148 (0.62)	1 (0.01)	82 (0.68)	37 (0.31)	16 (0.43)
Kolhorn	Open	168	165 (0.98)	1 (0.01)	2 (0.01)	160 (0.95)	2 (0.13)	7 (0.47)	5 (0.33)	0 (0.00)
			***	NS	***	***	*	NS	NS	NS
Robbenoordbos	Shaded (77 year)	161	91 (0.57)	3 (0.02)	67 (0.42)	61 (0.38)	8 (0.13)	23 (0.36)	33 (0.52)	16 (0.48)
Robbenoordbos	Open	278	153 (0.55)	51 (0.18)	74 (0.27)	118 (0.42)	19 (0.13)	79 (0.53)	49 (0.33)	7 (0.14)
			NS	***	***	NS	NS	*	*	***
Cirkelbos	Shaded (2 year)	158	127 (0.80)	31 (0.20)	0 (0.00)	87 (0.55)	39 (0.30)	49 (0.37)	42 (0.32)	18 (0.43)
Cirkelbos	Open	96	76 (0.79)	20 (0.21)	0 (0.00)	59 (0.61)	17 (0.26)	28 (0.42)	20 (0.30)	9 (0.45)
			NS	NS	NS	NS	NS	NS	NS	NS
Kathedralenbos	Shaded (22 year)	148	64 (0.43)	75 (0.51)	9 (0.06)	35 (0.24)	16 (0.13)	36 (0.30)	66 (0.55)	40 (0.61)
Kathedralenbos	Open	163	136 (0.83)	4 (0.02)	23 (0.14)	93 (0.57)	35 (0.30)	34 (0.29)	42 (0.36)	8 (0.19)
			***	***	*	***	**	NS	**	***
Wilgenreservaat	Shaded (36 year)	216	182 (0.84)	31 (0.14)	3 (0.01)	46 (0.21)	25 (0.15)	3 (0.02)	135 (0.81)	40 (0.53)
Wilgenreservaat	Open	332	253 (0.76)	77 (0.23)	2 (0.01)	65 (0.20)	41 (0.18)	2 (0.01)	184 (0.80)	8 (0.19)
			*	*	NS	NS	NS	NS	NS	***

Abbreviation : NS, not significant.

The estimated age (in years, ±3 year) at the time of sampling is given for the shaded habitat. Yellow (eU) comprises all yellow shells with bands 1 and 2 missing. Frequencies of banding categories are given as the proportion of all banded snails. For rarer banding categories, see Supplementary Table 1. Band fusion is calculated as the number of 5-banded snails with at least two bands fused. Statistical significances (χ^2-test): NS $P>0.05$; *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Zuiveringsveld and Cirkelbos) do the open and shaded samples of a pair cluster together. Also, there is a slight indication of area effects at the scale of polders; except for Dijkgatsbosch Open, the WI samples all fall within one branch of the phenogram. Similarly, the frequency of B appears to be low in ZF compared with the other polders (Table 1).

The morph frequencies (Table 1) show that most habitat pairs differ significantly for three or more morphs, except for Cirkelbos and Zuiveringsveld. However, most morphs do not show a consistent habitat pattern. In shaded habitats, there seem to be tendencies for Y and 12345 to increase, and for P and 00300 to decrease. Other morphs show about as many increases as decreases, or show no differences. The only consistent response is in fusion: all seven significant changes in band fusion are towards more fusion in the shaded habitat. The lack of a consistent response in other shell morphs is also apparent in the Cain and Sheppard diagram (Figure 3). When shell colour is converted to a general darkness score, however, patterns become clearer (Figure 4). In nine cases
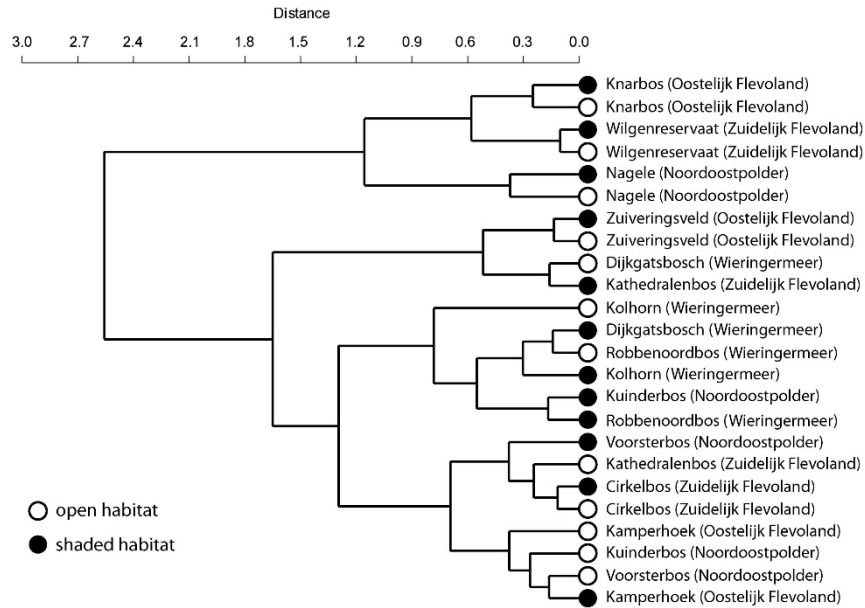
Figure 2 Phenogram of all samples, based on a (Ward-method) cluster analysis for euclidean distances among all morph frequencies.
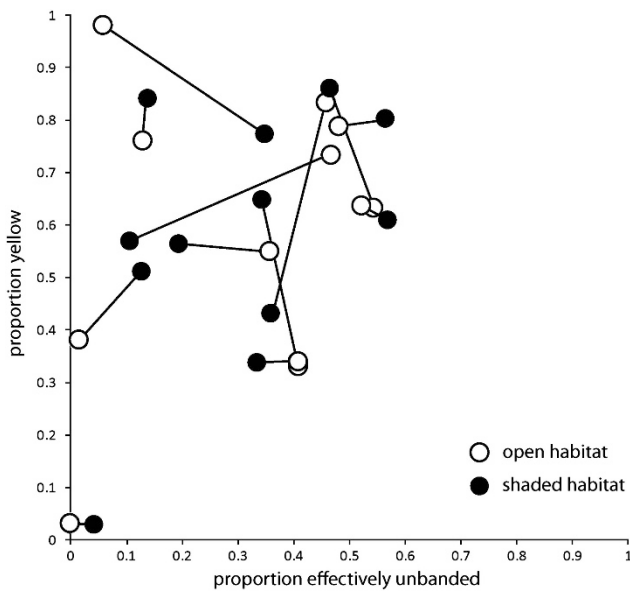

Figure 3 Cain and Sheppard diagram for all paired samples.

(seven of which are significant), shells in shaded habitats have a higher darkness score, and in only three (two significant) the pattern is reversed. The overall pattern of a change toward darker shells in shaded habitats is significant ($P < 0.001$; Fisher's combined probability test; $\chi^2 = 115$; d.f. = 24). Finally, open vs shaded habitat morph frequency difference shows positive and significant correlation with habitat and area age (Figure 5), leading to mean evolutionary rates of 14.7 (s.d. 20.7) and 31.4 (s.d. 45.2) kilodarwin (using polder age and habitat age, respectively).

DISCUSSION

Upon reclamation, the soil that developed in the IJsselmeerpolders provided a suitable habitat for terrestrial mollusks because of calcium-rich marine clay (Jansen, 2010). It may thus be expected that open habitats suitable for *Cepaea* began to appear naturally very soon after

reclamation was completed. Shaded habitats also appeared quickly, as forest was planted from saplings grown for this purpose at nurseries either within the polders themselves or on the 'old land' (Reinink, 1979). However, it appears that *Cepaea* was not introduced to the polders during this afforestation process, and in fact, only appeared many years after reclamation. In 1959, the species could not be found in grassland, woodland, nor the tree nurseries in OF, 2 years after reclamation (Jansen, 2010). Blaauw (1974), who surveyed land snails in ZF 5 years after reclamation, also records *Cepaea* as absent. Finally, Reinink (1979) failed to find *Cepaea* in ZF and OF in 1973/1974 (5–6 and 16–17 years after reclamation, respectively). He searched all tree nurseries and recently planted plots thoroughly and, finding no snails at all, concluded that the phytosanitary measures were very strict.

It thus appears that *Cepaea* has colonised the polders slowly and under its own power. It is therefore likely that it has entered planted woodlands after advancing through open habitat for several generations. More importantly, this means that any differences between populations in paired open and shaded habitats probably indeed evolved as these habitats appeared and that shaded habitats were not stocked with populations pre-adapted to woodland habitats. The pattern of colour morph variation in the polders suggests that these colonising populations retained sufficient genetic diversity to adapt to newly-arising environments, and also that some geographical structuring is present. In some cases, the populations from open habitat are, despite divergent evolution (see below), more similar in morph frequencies to the shaded habitat in the same locality than to other populations inhabiting open habitats.

The results show, for most localities, strong differentiation in several morph frequencies between open and shaded habitat (Table 1). When converted to a general darkness score, a significant tendency in the expected direction exists, namely towards darker shells in shaded habitats (seven significant cases), with a minority (two significant cases) showing the reversed response. Three cases (Nagele, Robbenoordbos and Zuiveringsveld) show no significant difference in darkness.

Absence of differentiation may be due to a rate of gene flow that is too great for differentiation to build up. In the case of Nagele, this is a
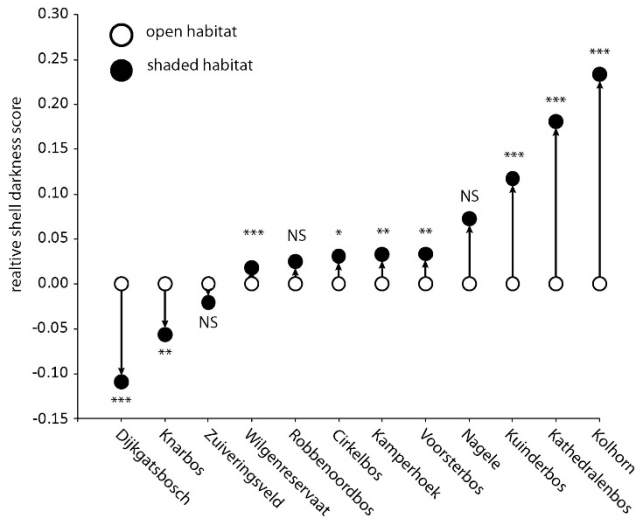
Figure 4 Diagram showing mean darkness scores for the shaded habitats compared with the corresponding open habitats (for convenience, darkness score set to zero for the open habitat). Statistical significances (Kruskall–Wallis one-way analysis of variance): NS $P>0.05$; *$P<0.05$; **$P<0.01$; ***$P<0.001$. The overall pattern of a change towards darker shells in shaded habitats is significant ($P<0.001$; Fisher's combined probability test).
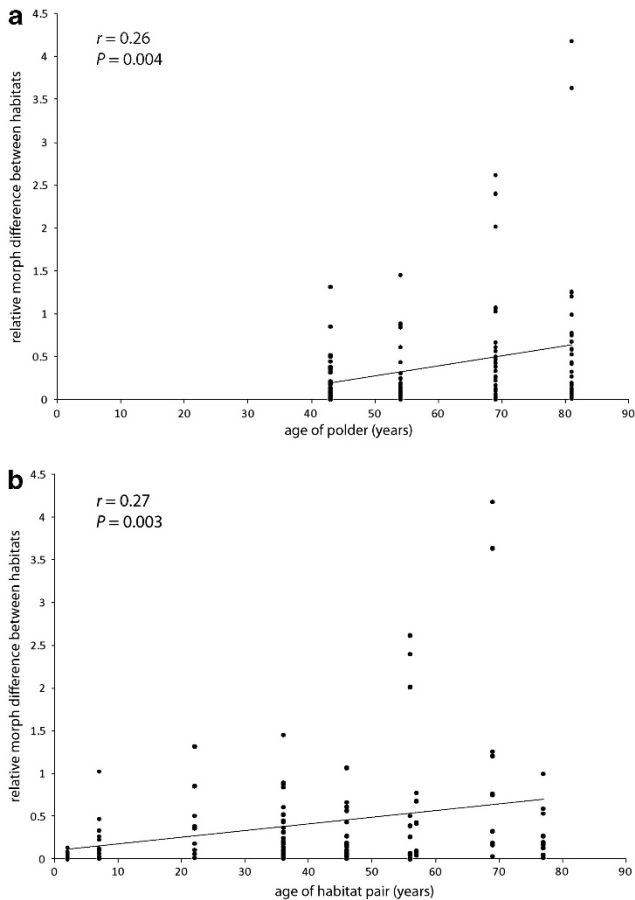

Figure 5 Relative frequency differences in all major morph categories for open vs shaded habitat for each locality, plotted against polder age (**a**) and estimated age of the shaded habitat (**b**). Significance of correlation coefficient (*r*) was assessed with F-tests.

plausible explanation, as the open habitat is a narrow strip of reedland immediately adjacent to the woodland (at a distance of the same magnitude as the per-generation dispersal in *C. nemoralis*; Jones *et al.*, 1977). At Robbenoordbos and Zuiveringsveld, however, this cannot be the case, as the two habitats, which have existed for several decades, are separated by 650 and 180 m, respectively, and, in both cases, by a road and two ditches. It is therefore not clear what may have prevented differentiation at these two localities.

The pattern for the remaining localities, of a majority of cases with darker shells in shaded habitat and a minority of cases with a reversed pattern, has been encountered before (Ożgo, 2005; Cook, 2008; Cameron and Cook, 2012a). It is generally assumed that the combined effects of visual selection and thermal selection tend to work in the same direction, that is, towards darker shells in the shaded habitat, but may sometimes work in opposite directions, depending on the alleles present in the population, the exact vegetation structure (which differed within the open and shaded habitat categories), and the season in which most selection takes place (Jones *et al.*, 1977). As a consequence of the relative strengths of thermal and visual selection pressures, this may sometimes lead to lighter shells in the shaded habitat.

Nonetheless, the majority of the IJsselmeerpolder localities show darker shells in the shaded habitat. The shell morphs responsible, however, differ strongly per locality. For example, at Kathedralenbos, yellow is decreased dramatically in favour of pink. At Kolhorn, yellow is also decreased, but here in favour of brown. At Voorsterbos, yellow is actually increased, but this is more than compensated by a greater number of banded snails. The only morph change that seems to be consistent across all localities is towards more band fusion in the shaded habitat. A similarly predictable response of band fusion was also found by, for example, Ożgo (2011). In fact, surprisingly, the categories most often reported to show consistent responses to habitat, that is, yellow and effectively unbanded, show no indication of a consistent response (Cain and Sheppard diagram; Figure 3). Cameron and Cook (2012b) similarly found that the habitat separation in a Cain and Sheppard diagram is only clear in Southern England and disappears on large parts of the European mainland. As changes in these colour morphs are most often thought to be associated with visual selection, it may be argued that the evolutionary changes that we do see in the present study are caused primarily by thermal selection, rather than visual selection. In this respect, it must also be pointed out that the inheritance and heritability of band fusion (the trait that contributes most consistently to shell darkness in this study) is far from clear (Lang, 1904; Murray, 1975), and further experiments to elucidate this are needed.

The chief aim of this paper was to examine the rate of evolutionary change, by making use of the known ages of the polders and the habitats in them. Interestingly, significant positive correlations exist between morph frequency differentiation and age, both for the age of the entire polder and for the respective habitats. Ages are given in years, but as generation time in *C. nemoralis* is *c.* 3 years (Jones *et al.*, 1977; Cook, 1998), the range equates with a time scale of 1–25 snail generations. Over this time scale, evolutionary change in shell colour traits appears to accumulate with a mean rate of 15–31 kDar, and Figure 5b suggests that this process has not yet stabilised. It should be emphasised that these are minimum rates: the (limited) data on colonisation history suggest that the polders were not colonised immediately, and, hence, snail populations may, in some cases, have been established considerably later than the polders and/or the habitats, or there may have been a history of extinction and recolonisation. It should also be emphasised that the evolutionary

change is not just the result of habitat-related selection, but probably include other kinds of genetic differentiation (for example, founder events and genetic drift) as well. Future work on comparisons of genetic differentiation across the same habitats in different polders and vice versa might enable separation of these multiple effects.

The rates of evolutionary change found here may be considered high (Kinnison and Hendry, 2001; Bell, 2008), and they clearly show that, indeed, as reported by Ożgo (2011), Ożgo and Kinnison (2008) and Ożgo and Bogucki (2011), *Cepaea* is capable of very rapid adaptive divergence in shell colouration in response to different habitat conditions. At the same time, the fact that evolutionary divergence seems to be greatest in those habitat pairs that have existed around 80 years (27 generations), it is not impossible that divergence will reach equilibrium only on a time scale close to 100 generations, as suggested by Cook (1998).

DATA ARCHIVING
Data have been deposited at Dryad: doi:10.5061/dryad.6d86v.

CONFLICT OF INTEREST
The author declares no conflict of interest.

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