

Evolutionary response of the egg hatching date of a herbivorous insect under climate change

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Under changing climatic conditions, species need to adapt to their new environment. Genetic adaptation is crucial to prevent population extinction¹ but examples where climate change leads to genetic changes in wild populations have been few^{2,3}. The synchronization between the timing of egg hatching of a herbivorous insect, the winter moth (*Operophtera brumata*), and the seasonal bud burst of its food plant, oak (*Quercus robur*), has been disrupted by climate change⁴ and a quantitative genetic model predicts that selection will delay the egg hatching date⁵. Here we show, using both long-term observational data and experiments, that the egg hatching date has changed genetically, resulting in closer synchrony with oak bud burst. The observed rate of change matches the predicted rate of change of one day per year. Hence, altered selection pressures, caused by environmental change, result in a rapid adaptive response in insect phenology. These genetic changes in a key life-history trait in this herbivorous insect therefore seem to be fast enough to match the climate-change-induced advancement of their host phenology.

Many organisms now face rapidly changing environmental conditions, due to anthropogenic changes in climate or land use^{6–8}. A major challenge is to predict the rate at which populations will be able to adapt¹. If this rate lags behind the rate at which the environment changes, this may have major consequences for population viability^{1,7,9}. One way of coping with changes in the environment is phenotypic plasticity—that is, the ability of one individual (genotype) to express different phenotypes depending on the environmental conditions¹⁰. Phenotypic responses to changes in climate are relatively often reported and include changes in phenology, that is, the natural timing of events, such as plant leafing^{11–13}, bird egg laying and migration¹³ and butterfly emergence^{14,15}. Phenotypic changes are however often not sufficient to match the changes in the environment^{16–19}. If species are to adapt to a new set of environmental circumstances, the phenotypic response to environmental stimuli, that is, the reaction norms¹⁰, needs to change genetically^{1,16}. Examples of changes in genotype distributions (micro-evolution) are much rarer than reports on phenotypic change^{2,3}.

Here, we investigated the changes in the winter moth (*O. brumata*) egg hatching date in response to temperature. Winter moths have an annual life cycle where adults emerge from their pupae in winter (November and December), mate and the females lay eggs on the branches of host trees. In spring (April and May) the eggs hatch and the emerging caterpillars need young foliage to feed on. Leaves from deciduous trees are suitable during only a short period of time; hence, the timing of egg hatching relative to bud opening is crucial²⁰. Even a few days' difference between egg hatching and oak

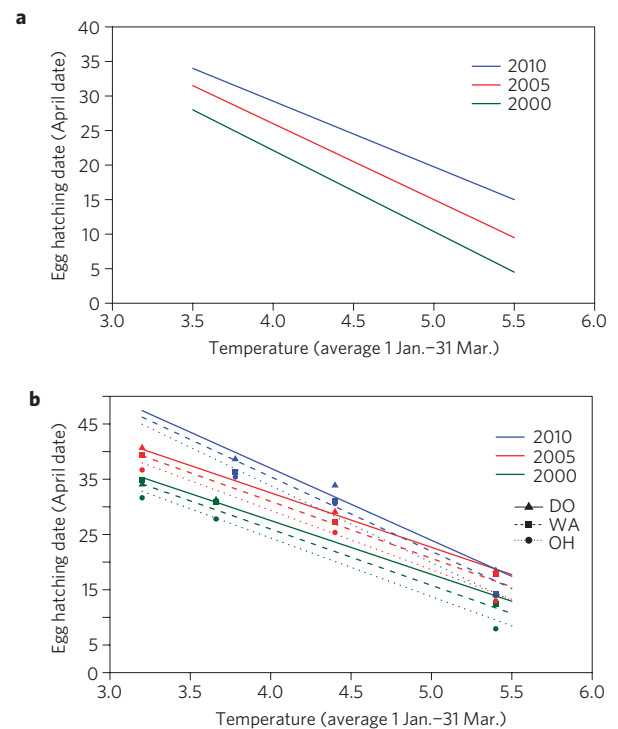


Figure 1 | Predicted and observed changes in the winter moth egg hatching date. a, Predicted changes in the reaction norm of the winter moth egg hatching date (in April dates, 1 April = 1; 1 May = 31) versus temperature (2000–2010, using the model in ref. 5). **b**, Observed changes in reaction norms from a reaction norm experiment (carried out in 2000, 2005 and 2010) in three areas (DO, OH and WA). In two cases the actual temperatures deviated: in 2000 the intermediate temperature treatment was too low and in 2010 the temperature in the colder treatment was warmer than intended.

(*Q. robur*) bud opening has marked fitness consequences^{20–22}. Both oak bud opening and winter moth egg hatching are temperature dependent, and as temperatures have increased, both have advanced over the past decades¹. Winter moth egg hatching has however responded more strongly to the increase in temperature, leading to a decrease in the synchronization between oak and winter moth in the Netherlands (but see ref. 23 for an English population). In this case the phenotypic response of the egg hatching date to temperature is not sufficient to remain in synchrony with the host plant. Therefore,

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Table 1 | Analysis of winter moth egg hatching date in a split-brood design experiment where winter moth eggs from three areas (OH, DO and WA) are kept under different temperatures to assess egg hatching reaction norms (see also Fig. 1b).

	numDF	denDF	F value	p value
(Intercept)	1	435	10,248	<0.001
Year	2	219	9.21	<0.001
Area	2	219	19.8	<0.001
Temperature	1	435	9,936	<0.001
Area × temperature	2	435	11.3	<0.001
Year × temperature	2	435	80.6	<0.001
Removed from final model				
Year × area	4	215	1.07	0.368
Year × temperature × area	4	431	1.74	0.139

genetic change of the plasticity itself is needed. Thus, adaptation is possible only if the elevation and/or the slope the reaction norm of egg hatching versus temperature changes genetically. We expect the egg hatching date to become later, for a given temperature, as the eggs now hatch too early⁵.

Given present selection pressures and estimates of genetic (co)variation in the reaction norm⁵, we could make predictions at which rate the reaction norm should change. A quantitative genetic model (described in ref. 5; see also Methods) predicted that the

elevation of the reaction norm should have increased from 2000 to 2005 by 3–6 days (Fig. 1) and the largest change should have been at the higher temperatures (change in slope of the reaction norm). We have been studying the same populations from 1995, and therefore we could compare the predictions of the model, which we here updated to 2010, with the actual, observed changes in the reaction norm of egg hatching versus temperature over the period 2000–2010.

Results

Predicted genetic changes in winter moth reaction norms. At the start of the long-term field study (1995), eggs tended to hatch one to two weeks before the oak buds opened and thus before the food became available to feed on. This was most pronounced in warm springs, with occasionally eggs hatching up to three weeks before the oak leafing started^{2,4}. This led to strong selection for later egg hatching and consequently our model predicts a change in the reaction norm, mainly in elevation, with eggs predicted to hatch about 7–10 days later in 2010 than in 2000, but also with the largest change at warmer temperatures, leading to a shallower reaction norm (Fig. 1a).

Observed genetic changes in winter moth reaction norms. We used two different approaches to test the predicted changes in reaction norm elevation and slope (Fig. 1a). In our first approach we used an experimental set-up to determine whether the winter moth reaction norm has changed by using a split-brood design experiment where eggs were kept under different temperatures and

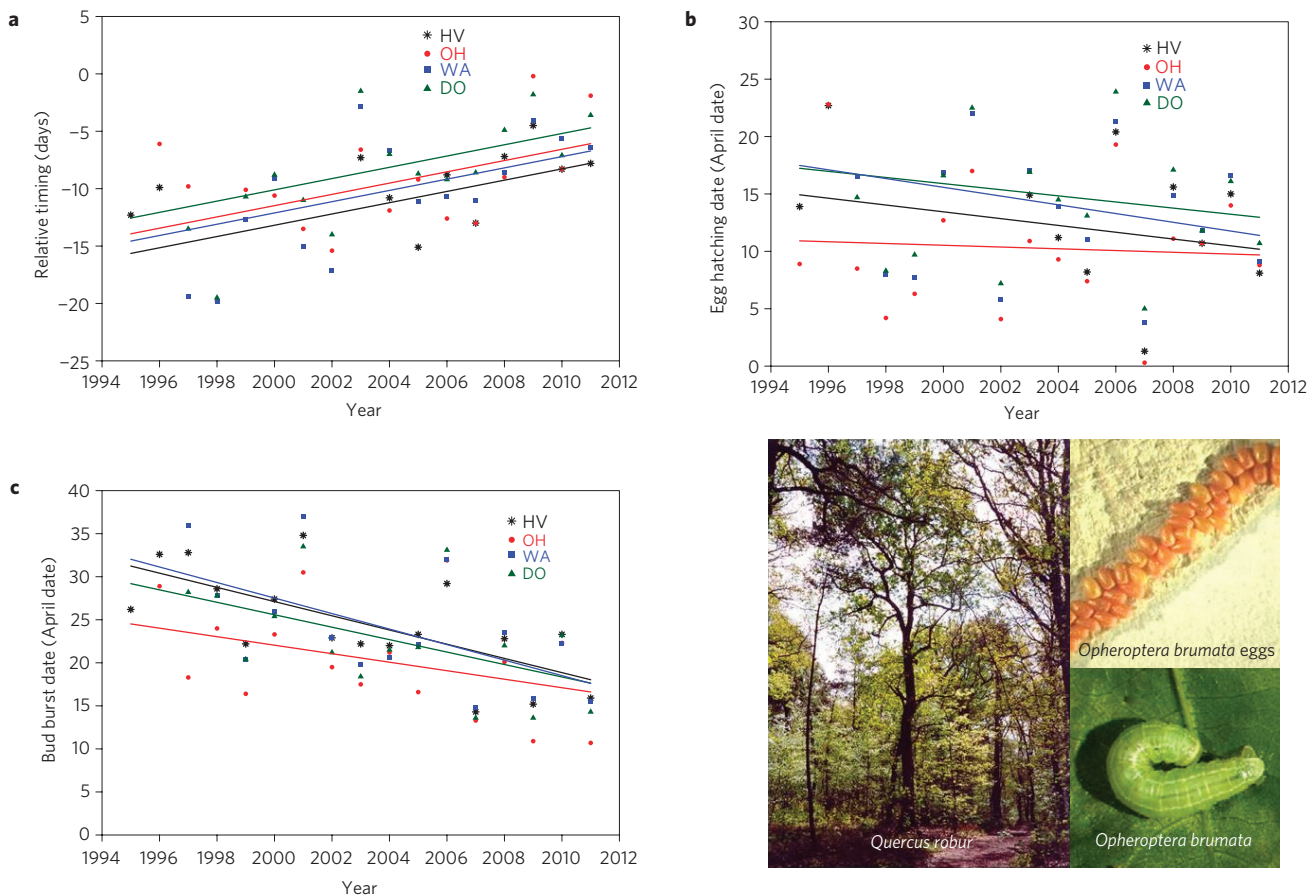


Figure 2 | Long-term field data (1995–2011) in four areas in the Netherlands. a–c, Observed changes over time in relative timing (days; **a**), winter moth hatching date (in April dates, 1 April = 1; 1 May = 31; **b**) and oak bud opening (April dates; **c**) in four areas (HV, OH, WA and DO). Relative timing is defined as the difference between oak bud opening and egg hatching dates (negative values indicate that eggs hatched before buds burst). Symbols represent observed data points; lines are the fitted model estimates (at a constant temperature of 4.5 °C).

Table 2 | Analysis of winter moth egg hatching date from a long-term field study (1995–2011) in four areas: DO, OH, WA and HV.

	numDF	denDF	F value	p value
Relative timing				
(Intercept)	1	37	159.0	<0.001
Year	1	14	9.48	0.008
Area	3	37	4.78	0.006
Temperature	1	14	5.73	0.031
Removed from final model				
Year × temperature	1	13	1.12	0.307
Year × area	3	34	0.53	0.659
Area × temperature	3	31	0.26	0.852
Year × area × temperature	3	28	1.14	0.348
Winter moth egg hatching date				
(Intercept)	1	29	228.1	<0.001
Year	1	13	0.72	0.409
Area	3	29	56.5	<0.001
Temperature	1	13	28.2	<0.001
Year × temperature	1	13	0.35	0.563
Year × area	3	29	3.50	0.027
Area × temperature	3	29	0.89	0.457
Year × area × temperature	3	29	4.62	0.009
Oak bud opening date				
(Intercept)	1	40	435.8	<0.001
Year	1	14	10.45	0.006
Area	3	40	15.29	<0.001
Temperature	1	14	4.81	0.045
Year × area	3	40	2.74	0.055
Removed from final model				
Year × temperature	1	13	0.07	0.789
Area × temperature	3	37	0.34	0.794
Year × area × temperature	3	34	1.31	0.285

Parameters both included and excluded from the final model are shown. For each forest each year represents one data point; relative timing is defined as the number of days between oak bud opening and egg hatching. NumDF and denDF indicate the degrees of freedom in the numerator and denominator respectively. See also Fig. 2 and Table 3 for the estimates.

for which we resampled females from the same trees on the same date in five-year intervals. In 2010 eggs hatched five to ten days later than in 2000 when kept under the same controlled conditions (Fig. 1b and Table 1). Although the eggs in general hatched later in recent years (increase in reaction norm elevation— Table 1: year $F_{2,219} = 9.21, p = 0.001$), this difference was most pronounced under the colder treatments (change in reaction norm slope— Table 1: year × temperature $F_{2,435} = 80.6, p < 0.001$). Eggs from Oosterhout (OH) always hatched earlier than those from Warnsborn (WA) and Doorwerth (DO; Fig. 1b).

Second, we use our long-term field data (1995–2011) to determine whether these changes in the reaction norm have led to a change in the timing of winter moth egg hatching relative to oak bud opening (Fig. 2a). Whereas in 1995 eggs tended to hatch one to two weeks before the oak buds opened, over the study period this timing difference has become smaller, meaning that the eggs hatched in closer synchrony with the oaks in more recent years (Table 2: $F_{1,14} = 9.48, p = 0.008$).

Timing depends not only on the phenology of moths, but also on that of oaks. In some areas and for some temperatures the winter moth eggs hatch slightly earlier nowadays (see Fig. 2b and

Table 3 | Estimates of an analysis of winter moth egg hatching date.

	Area	Temperature	Year	Year × temperature
DO	44.2 (±6.10)	−5.8 (±1.25)	−1.53 (±0.59)	0.27 (±0.12)
OH	27.8 (±4.35)	−3.7 (±0.85)	−0.37 (±0.37)	0.06 (±0.07)
WA	44.6 (±4.57)	−5.9 (±0.89)	−1.54 (±0.38)	0.25 (±0.07)
HV	26.6 (±4.65)	−2.6 (±0.97)	−0.26 (±0.38)	0.00 (±0.08)

Estimates calculated from a long-term field study (1995–2011) in four areas: DO, OH, WA and HV. Area and temperature estimates are the values at the start of the study period (1995), year and year × temperature estimates denote the change per year since then. See Table 2 for statistics.

Table 2: interaction year × area: $F_{3,29} = 3.50, p = 0.027$; interaction year × area × temperature: $F_{3,29} = 4.62, p = 0.009$. Over the study period, eggs from areas where oak bud burst occurs later, DO and WA, hatched later than those from OH and advanced their hatching date more at higher temperatures (Table 3).

Oak trees have advanced their oak bud opening during the study period by about a week on average (Table 2, $F_{1,14} = 10.45, p = 0.006$; Fig. 2c), with a trend for the late-developing areas to advance more than the earlier developing ones ($F_{3,40} = 3.74, p = 0.055$). This progressively earlier development has to be due to the temperature increase. Tree bud development is temperature dependent¹¹, and as the trees used in the study are the same throughout the whole study period it cannot have been caused by (genetic) changes in the tree population.

Discussion

Winter moths are under selection to hatch their eggs later in spring as, owing to climate change, they hatch their eggs too early to match the development of the oak leaves, on which the caterpillars feed^{4,24}. The prediction of a quantitative genetic model that winter moth eggs hatch later for a given temperature, that is that they genetically alter their reaction norm for egg hatching versus temperature, is confirmed both in an experimental study and in a long-term field study: eggs now hatch later for the same temperatures and are more in synchrony with oak bud burst in the wild. Demonstrating genetic change to adapt to climate change is very rare^{2,3} but of key importance as populations need to change genetically to keep in pace with climate change¹.

The rate of change in reaction norm elevation across the areas is 0.19 haldanes from 2000 to 2010 (OH: 0.17; WA: 0.21; DO: 0.20), which is high compared with those reported in other studies²⁵. Potentially, some of this change could be due to maternal effects, which we have shown to be important in winter moths²⁶, but to some extent we excluded these maternal effects with our experimental set up where female winter moths were collected on the same date every five years: as the eggs are laid in the next few days after catching, egg laying date, and hence photoperiod, was thus kept constant. This also coincided with the main emergence peak of adults each year, thus avoiding the use of relatively very early or late individuals that might (conditionally or genetically) form a different, non-random subset of the population between years.

Our findings are comparable to the only other example of an insect changing genetically in response to climate change, the pitcher plant mosquitoes. In this species the critical photoperiod for pupation was altered such that the insects now enter diapause later²⁷. In our study an altered sensitivity to photoperiod is not likely as there is no effect of photoperiod on egg hatching date (M.v.a. *et al.*, unpublished) and also because the slope of the reaction norm of egg hatching date to temperature changed, which cannot be due to altered sensitivity to photoperiod (which can only

alter the reaction norm elevation). Thus, it is the winter moth's response to temperature that has genetically changed.

The genetic change in reaction norm is mainly a change in elevation and to a lesser extent in slope (Fig. 1b). Whereas the quantitative genetic model predicts the largest shift at high temperatures, making the reaction norm slope shallower (Fig. 1a), the data from the repeated split-brood design experiment show that the largest delay in egg hatching occurs at lower temperatures, making the reaction norm steeper (Fig. 1b). We are however cautious in our conclusions on this discrepancy as in the 2010 experiment, owing to a technical problem, the low-temperature treatment had higher temperatures than intended and thus for that year we lack a response of the eggs to low temperatures.

When comparing the different forests in the long-term data set, the egg hatching dates in WA and DO are overall later and their reaction norms shifted more, both in elevation and slope, compared with the reaction norms for the Hoge Veluwe (HV) and OH (Tables 2 and 3). This can possibly be explained by the composition of trees within the forests. Although all four forests consist predominantly of mature oak trees, in both OH and HV other trees and shrubs such as maple (*Acer pseudoplatanus*) and red oak (*Quercus rubra*) are present underneath the oak trees. These may serve as an alternative food source for the larvae, thereby reducing selection (and thus genetic changes) within those forests. In WA, and to a lesser extent DO, there are hardly any alternative food plants, and thus the selection pressure is presumably stronger there.

Selection has led to genetic change in a key life-history trait in the winter moth, which has allowed it to get in better synchrony with the phenology of its food source, the oak's buds. One reason why the moths could adapt is that they have one generation each year whereas the oaks have a much longer generation time. As the present climate is predicted to change much more rapidly than ever before²⁸, the pivotal question is determining whether species can keep up with the rate of climate change, rather than whether or not a species can adapt¹. Therefore, establishing the rate of genetic change is crucial and although it is unlikely that this will be the case for many species, winter moths are an example of adaptation to climate change through genetic shifts within a few generations.

Methods

Predicted changes in winter moth reaction norm. First, we predicted how the winter moth egg hatching date would have changed. We used a simulation model described in ref. 5. In the model, the response (R) to selection was determined by the heritability (h^2 ; for example how much do offspring genetically resemble their parents?) and by the selection (S ; for example, which part of the population survives and, thus passes on their genes to the next generation?)²⁹: $R = h^2 \times S$.

Egg hatching is dependent on environmental cues, which differ between years. This means that the response to selection can differ between years, depending on the conditions in a given year. We were explicitly interested in changes in egg hatching across the different temperatures (the reaction norm). We therefore used a multivariate version of the equation³⁰: this gives the response across all possible environments in the next year to the selection in a given environment (x_i) in year i . We used a linear reaction norm to describe egg hatching. Both the intercept and the slope of the reaction norm can potentially change through selection. The change in the parameters of the reaction norm (Δg_{mean}) in a given year (i) was calculated by multiplying a generalized genetic covariance matrix (G_g) with a vector ($1 \times x_i$)¹ characterizing the environment (x_i) and with the selection gradient (β) in this year³⁰: $\Delta g_{\text{mean}} = G_g(1 \times x_i)^T \beta$. The selection gradient (β) depends on the mean trait value (z_{mean}) relative to the optimal population mean (z_{opt}) for a trait z . It also depends on the variation in the trait (z_s), as well as on the strength of selection (ws ; ref. 30): $\beta = -(z_{\text{mean}} - z_{\text{opt}})/(ws^2 + z_s^2)$. We then calculated the expected change in reaction norm in year i due to selection. This gives the new reaction norm, so that the process can be repeated, with new environmental $x_i + 1$ values and a new selection gradient, depending on the conditions in year $i + 1$. The genetic (co)variance matrix (G_g) was estimated using a half-sib experiment⁵. Fitness measurements were also experimentally obtained to determine the strength of selection (ws). For details on the model calculations see Supplementary Information.

Split-brood experiment to measure changes in winter moth reaction norm. We repeatedly determined the egg hatching date in response to different temperature

treatments (reaction norm) in three different years (2000, 2005 and 2010), to check experimentally whether the reaction norm has changed over time. Eggs of female winter moths were divided over three temperature treatments (at least 25 eggs per treatment per female, 14–30 females) (see Supplementary Information). In two cases the actual temperature in a treatment deviated from the intended one: in 2000 the intermediate temperature treatment was consistently too low (on average 3.7 °C instead of 4.4 °C), and in 2010 the temperature in the colder treatment was warmer than intended (average temperature 3.8 °C instead of 3.2 °C). All other temperature treatments were within ± 0.2 °C of one another, and we assumed that these were therefore identical.

Several factors affecting egg hatching can differ in the three years. To control for (genetic) within-year differences, we used eggs from females caught around the peak adult emergence date and from the same trees in each year. This prevented the use of different subsets of the population in the different years (that is, early-emerging females in one year and late-emerging ones in the other year). In 2000 and 2005, the mean catching date was 29 (± 0.9 s.e.m.) of November; in 2010 the mean catching date was 28 (± 1.0 s.e.m.) of November.

Analyses were done using a linear mixed-model procedure in *R* (version 2.12.2). Year and area of origin were the factorial explanatory variables, and the mean temperature from 1 January until 31 March in each treatment was a continuous explanatory variable. Clutch was included in the model as a random factor.

Long-term data to measure changes in winter moth reaction norm. We also collected field data on both oak bud opening date and winter moth egg hatch date during seventeen years (1995–2011) in four forests all in the area around Arnhem, the Netherlands (OH (51° 55' N, 05° 50' E), DO (51° 59' N, 05° 48' E), HV (52° 05' N, 05° 48' E) and WA (52° 05' N, 05° 50' E; see Supplementary Information). We calculated the degree of synchronization as the (average) bud opening date minus the (average) egg hatching date, in each year for each forest separately.

Analyses were done using a mixed-model procedure in *R* (version 2.12.2), with year and area of origin as explanatory variables. Temperature was also included (mean from 1 January until 31 March). To correct for the fact that we have multiple non-independent observations within a year, we also included year as a random factor.

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Author contributions

M.E.V. and L.J.M.H. set up the long-term field data collection, M.v.A. and M.E.V. designed the experiment, M.v.A., L.S., L.J.M.H., B.v.L. and M.E.V. collected the long-term field data, M.v.A., L.J.M.H. and B.v.L. carried out the experiment, M.v.A. and M.E.V. analysed the data and M.v.A., L.S. and M.E.V. wrote the paper.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to M.E.V.

Competing financial interests

The authors declare no competing financial interests.