Insect Conservation and Diversity

Insect Conservation and Diversity (2010) **3,** 44–50 doi: 10.1111/j.1752-4598.2009.00071.x

Measuring evolutionary responses to global warming: cautionary lessons from *Drosophila*

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Abstract. 1. Understanding evolutionary responses to global climate warming can be dauntingly complex. But, primarily, it requires careful quantification of rates of temporal change of biomonitors.

2. Long-term biomonitoring programs capitalize on traits for which there already exist retrospective measurements. Those programs are thus faced with the decision as to the appropriate time to update historical records, especially in species whose gene frequencies cycle over the successive seasons. In these cases, the time during the year at which descendant populations are sampled needs to take into account effects of the lengthening growing season that can impose direct selection on specific genotypes and, concomitantly, indirect selection on photoperiodic response.

3. Standardising new and past collections by calendar date can lead to an overestimation of the magnitude of long-term responses to global warming; standardising by equivalent seasonal temperature conditions can lead to an underestimation of the magnitude of long-term responses to global warming.

4. The results of a recent monitoring study with *Drosophila* indicate that caution should be exercised in how the updating of historical records is used to quantify evolutionary responses to global warming.

Key words. Bioindicators, chromosomal inversions, climate change, historical records, molecular markers, photoperiod, seasonal selection, seasonality, thermal selection.

Introduction

Twentieth-century global climate warming of approximately 0.6 °C has already affected the Earth's biota. This relatively mild level of thermal increase has elicited shifts in species' ranges towards the poles and higher elevations, altered phenological patterns, and adaptive population genetic changes (Parmesan, 2006). Idiosyncrasies as to the direction and magnitude of species' responses are indirectly driving many biotic interactions increasingly further out of synchrony, threatening the stability of existing ecological assemblages. A major challenge now is to predict, how biological impacts of climate change will unfold in response to projected temperature increases of up to 6 °C by 2100 (Intergovernmental Panel on Climate Change, 2007).

Understanding the trajectory of biological processes under global warming begins by obtaining accurate estimates of the biotic responses that are actually taking place. Estimates of ongoing climate warming effects are increasingly obtained using long-term field monitoring programs (Hughes, 2000; Parmesan, 2006; Hoffmann & Daborn, 2007; Schwartz *et al*., 2007). Those programs typically seek to reconstruct continuous ecological and evolutionary processes from sparse measures, taken only at specific times. Long-term monitoring surveys capitalize on diagnostic features – bioindicators or biomonitors – for which there are historical data available.

Updating historical records is proving to be far less straightforward than might at first have been supposed. Several authors have uncovered difficulties associated with the criteria for data selection, and sampling and statistical methodologies (Parmesan, 2007). A different type of problem arises from inaccuracies as to the precision with which time-reckoning systems track the course of changes to the Earth system. An early warning came with the realisation that the Gregorian calendar year (365.2500 days, or the average over three ordinary years plus one leap year) lasts 11'14" longer than the vernal equinox year (365.2422 days, or the interval between one vernal equinox and the next) (Sagarin, 2001). This difference causes calendar dates to lag behind vernal dates. An analysis of published long-term studies of phenological trends, showed that neglecting the delay

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in the calendar date of the vernal equinox can introduce a bias towards stronger values of earlier spring, hence to overestimating the magnitude of global warming effects on the onset of spring – up to 10% in some of the longest records (Sagarin, 2001). Herein, we critically assess recent evidence of the problems that can arise at measuring long-term directional genetic responses to global warming from updates of historical records of genetic markers that also track the seasonal climatic cycle, as well as strategies to handle them.

Quantifying long-term evolutionary responses in the presence of a non-stationary seasonal cycle

Fitness in seasonal environments reflects the ability of individuals to exploit the favourable season, to mitigate the effects of the unfavourable season, and to make the timely switch from one life style to the other. Traits involved in climatic adaptation are likely to covary with the seasonal climatic cycle. Candidates for monitoring biological effects of climate warming are expected to be enriched in traits undergoing seasonal cycling. However, seasonal cycling introduces a key difficulty for updating historical records, which ensues from the need to decide on the most appropriate time to carry out the update.

In univoltine and multivoltine species, particularly in the latter with shorter generation times relative to season length, the seasonal climatic cycle can induce constantly recurring seasonal changes in climate-related traits. Under strict cyclical seasonal selection, the selective differences in the alternating seasons would cancel each other at the completion of the annual cycle (Hedrick, 1976; Hoekstra & Van Delden, 1978). Seasonal cycling can be erroneously interpreted as a long-term response to climate warming when observations are made at different times of the annual cycle in different years. The natural approach in order to prevent confounding of long-term trends with short-term seasonal cycles is to collect updates on the same calendar dates as the historical records. The validity of this approach depends fundamentally on the premise that the environmental seasonal cycle has remained stable over the monitoring period (Fig. 1). However, a burst of studies over the past few years clearly show that this premise is untenable.

One of the most prominent phenomena brought on by the globally rising greenhouse gas concentrations is a nearly universal trend towards a lengthening of the growing season. The rate of this lengthening has accelerated in the last three decades, particularly at higher, more seasonal latitudes and/or regions where the temperature rise has been steepest (Badeck *et al*., 2004; Dose & Menzel, 2004). The lengthening has progressed asymmetrically, being more strongly marked at spring (2.3–2.8 days advance decade^{-1}) than at autumn (1.3 days delay decade^{-1}) (Linderholm, 2006; Cleland *et al*., 2007), apparently because climate warming is proceeding chiefly through warming of the coldest days in late winter and early spring, rather than consistently throughout the year (Cleland *et al*., 2007; Parmesan, 2007).

Because of the increase in the length of the growing season, present calendar dates, particularly those from spring and – less so – autumn tend to be warmer than their corresponding historical dates. If the target traits cycle seasonally, then updating historical records on the same calendar dates would incur a bias towards obtaining an enhanced summer/low-latitude like trait configuration, compared to that expected from the long-term component of the global warming induced selection alone (Fig. 1). Inferred responses would be maximally skewed if the adaptive differences imposed by the novel seasonal patterns cancel out each other at the end of every annual cycle. The problem will worsen as the growing season continues to become longer.

Monitoring *Drosophila* inversion polymorphisms: a case in point

Drosophila inversion polymorphisms are among the first genetic markers quantified in natural populations (Dobzhansky, 1937; Powell, 1997; Hoffmann *et al*., 2004). There is a great deal of historical data to investigate effects of global environmental changes by comparison with current configurations. An illustration of this potentiality, and the problems of uncritically updating historical records is provided by the temperate zone, multivoltine *Drosophila subobscura*.

Research on the inversion polymorphisms of *D. subobscura* began well before global warming became a major concern. Early in the field studies, they were found to vary clinally along the 30° latitude spanning the fly's native Palearctic range. Following the recent introduction of the species to the New World, several inversions converged independently in North and South America to their respective clines in the Old World (Balanyà *et al*., 2006). Some clinal arrangements undergo seasonal cycles, which occur as it would be expected from their corresponding clines if they were caused by temperature (Rodríguez-Trelles *et al*., 1996). Persistence of the spatiotemporal patterns despite continent-wide gene flow further attests that they are adaptive (Balanyà *et al*., 2006).

A time series analysis (four seasonal samples per year, collected during two 4-year periods separated by ~one decade) of *D. subobscura*'s O chromosomal polymorphisms in northwest Iberia started in the mid-1970s, coinciding with the onset of the second warming period of the 20th century, detected an increase in the incidence of warm-associated inversions – with a decrease of cold-associated inversions, and a 18% loss of chromosomal diversity. The study did not explicitly consider the lengthening of the growing season in the sampling schedule. Consistency of the inter-annual shifts in inversions frequencies across the four monitored seasons; however, attests that the observed patterns represent genuine long-term directional trends, rather than short-termed plastic/genetic responses to a seasonal event. Similar patterns were observed at other distant Palearctic localities, which bolstered the hypothesis that fly populations are evolving in response to global warming (Rodríguez-Trelles & Rodríguez, 1998; Rodríguez-Trelles *et al*., 1998). This view has since been corroborated using this and a few other widespread *Drosophila* species, by research conducted before current awareness of the lengthening of the growing season (Anderson *et al*., 2003; Balanyà *et al*., 2004; Levitan & Etges, 2005; Umina *et al*., 2005).

The hypothesis was recently revisited using updates of historical records (Balanyà *et al*., 2006). This interesting survey

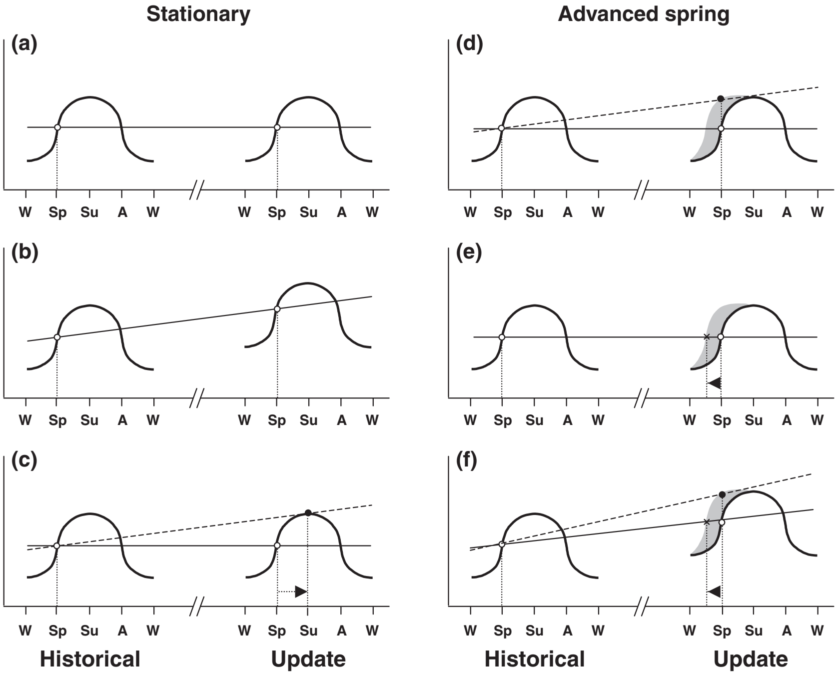

Fig. 1. Six hypothetical scenarios (a–f) showing how neglection of seasonal cycling at scheduling updates of historical records of an indicator can lead to biased inferences of long-term effects of global warming. If the seasonal cycles of the indicator are stable (a–c), estimation of long-term variation (solid lines in a and b) requires only standardisation of new and historical collections by calendar date (the same spring date in a and b). Trends obtained from updating on a different date (a summer date in c), can be spurious (dashed line in c). If the seasonal cycles of the indicator are non-stationary, like in the current situation of global warming induced progressive advancement of spring (shaded area in d–f), artifactual long-term trends (dashed lines in d and f) can result from updating the same calendar date as historically (black dot in d and f). The bias can be prevented by standardising new and historical collection dates by seasonal climatic conditions (cross-marks and solid lines in e and f). W: winter; Sp: spring; Su: summer; A: autumn.

considered all five acrocentric chromosomes of *D. subobscura* at 26 locations along a latitudinal gradient in the Old World, and North and South America, 22 of which warmed over the median 25 years of the sample interval. For most sites (18), the historical data came from spring, when the bias introduced by not taking into account the lengthening of the growing season in the sampling schedule is expected to be greatest. Inversion frequencies were pooled into a chromosomal index, rather than analysed individually. Comparison of the historical with the updated record at each site showed a shift towards enhanced low-latitude values of the index on the three continents, which was correlated with the warming underwent by the locations. The congruence of individual inversion patterns across continents was not assessed. The result was construed in line with previous claims of long-term evolutionary responses to global warming.

Alternatively, however, the estimated long-term shifts could at least in part be overestimates, since the survey overlooked the lengthening of the growing season in the re-sampling scheme (Rodríguez-Trelles & Rodríguez, 2007). On one hand, *D. subobscura* inversions cycle seasonally, at least in some populations (Rodríguez-Trelles *et al.*, 1996). Seasonal cycles of inversions have been reported for other *Drosophila*, including *D. pseudoobscura* (Dobzhansky, 1943), *D. persimilis* (Dobzhansky, 1956), *D. funebris* (Dubinin & Tiniakov, 1945), *D. flavopilosa* (Brncic, 1972), *D. robusta* (Levitan, 1973; Etges, 1984), *D. melanica* (Tonzetich & Ward, 1973), *D. melanogaster* (Stalker, 1980; Sánchez-Refusta *et al.*, 1990), and *D. mediopunctata* (Ananina *et al.*, 2004). The aforementioned monitoring of an Iberian population of *D. subobscura* shows that the warm phase of the annual cycle of inversions underwent a rapid expansion parallelly with the lengthening of the warm season at the locality (Fig. 2). On average, spring mean temperatures (over the 30-day period before each collection date; Rodríguez-Trelles & Rodríguez, 1998) occurred 12.9 days earlier in the second (i.e. 1988–1990) compared to the first (i.e. 1976–80) sampling period. Were one to quantify the long-term behaviour of the inversion of Fig. 2 by re-sampling in the same calendar early-summer date, one would conclude that it became ~20% more frequent over the monitoring, when in fact, no change in its average inter-annual level was detected (Rodríguez-Trelles *et al.*, 1996; Rodríguez-Trelles & Rodríguez, 1998). On the other hand, the positive covariation between the Balanyà *et al.*'s chromosomal index and the average temperature of the sampling month (Balanyà *et al.*, 2007) indicates that the frequencies of low-latitude inversions tend to peak in summer. When four updates collected 1–2 months nearer mid-summer with respect to their historical counterparts are removed from the dataset, the degree of parallelism between the chromosomal and temperature shifts

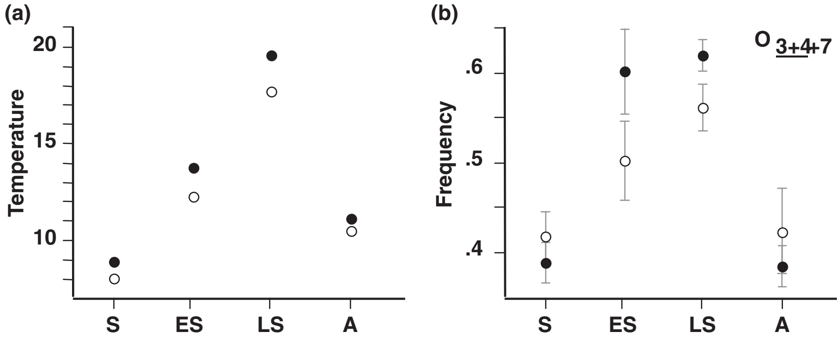

Fig. 2. A rapid genetic response to the expansion of the growing season. (a) Mean seasonal temperatures (calculated over the 30-day period before each collection date), and (b) average seasonal frequencies of $O_{\underline{3+4}+7}$, a southern chromosomal arrangement of *D. subobscura*, at northwest Iberia, in each of two tracking periods, namely 1976–1980 (open circles) and 1988–1991 (solid circles) (Fontdevila *et al.*, 1983; Rodríguez-Trelles *et al.*, 1996; Rodríguez-Trelles & Rodríguez, 1998). There is an increase in the warm season frequencies of the inversion [significant interaction period × season ($F_{1,27} = 5.11$, $P = 0.031$) in a two-way ANOVA grouping angularly transformed data into cold (S and A data) and warm (ES and LS) seasons] from the first to the second tracking period. S: spring; ES: early-summer, LS: late summer; A: autumn. Error bars are 1SE.

drops by ~20% (Rayleigh statistic of uniformity decrease from 0.78 to 0.60; Balanyà *et al.*, 2007), which demonstrates an effect of the season of re-sampling. The actual magnitude of this effect is probably greater, since the above reanalysis still included nine updates collected at the same calendar date as the historical records. These findings do not lessen the biological significance of Balanyà *et al.*'s corroboration of a global warming evolutionary response, but highlight the importance of accounting for the lengthening of the growing season in scheduling updates of historical records.

Seasonal climate or photoperiodism: is there an updating standardisation dilemma?

For the remaining 13 of the 22 comparisons considered in Balanyà *et al.* (2007), the updates were collected in a cooler month than retrospectively. Those updates were thus collected systematically in shorter daylengths, which raises the question of whether the reported chromosome shifts reflect differences in the photoperiodic environments between old and new dates, instead of a long-term response to global warming. In other words, will standardising new collecting dates by seasonal climate destandardise photoperiodic cues?

The annual photoperiodic cycle is the progression of day/night lengths caused by the rotation of the earth around the sun. Its predictability is exploited by many organisms as a cue to anticipate the seasonal change. Particularly relevant is the critical photoperiod, or daylength used to switch to the hibernal state, which is generally abrupt (Bradshaw & Holzapfel, 2007). It should be pinpointed that daylengths are 'fixed' cues, whereas critical photoperiods ensue from how individuals 'gauge' those cues. Evaluation of daylength regimes integrate inputs from a wide range of other abiotic and biotic conditions, including chiefly temperature. They are those background conditions, not local daylength *per se*, which impose selection on the optimal time to switch to the appropriate phenophase. In the present situation of sustained lengthening of the growing season, there is an expanding mismatch between photoperiodic cues and the optimal timing of seasonal events.

Sticking to anachronical photic cues can imply experiencing novel seasonal environments in suboptimal metabolic conditions, and/or mistimed with respect to key ecological interactions (Parmesan, 2006; Both *et al.*, 2009). A number of studies on the genetics of the photoperiodic response found it to be a high heritability trait, with capacity for a rapid adaptive response (reviewed in Bradshaw & Holzapfel, 2006, 2007). Just as latitudinal variation in climate has resulted in genetically determined latitudinal clines in photoperiodic response, so also will increasingly longer growing seasons select for adaptive genetic shifts in photoperiodic response. The evolutionary potential of the photoperiodic response has been investigated in connection with global warming (Bradshaw & Holzapfel, 2001, 2004). Over a period of 24 years, northern populations of the pitcher plant mosquito, *Wyeomyia smithii*, evolved a 0.6 h shorter critical photoperiod to enter winter diapause – corresponding to 9 days later in the fall – in response to directional selection imposed by the increasing longer growing season. Standardising new samples by seasonal climatic conditions should concomitantly take into account bias that may be imposed by shifting photoperiodic response. As to *D. subobscura*, most efforts carried out so far suggest that it does not undergo diapause, its seasonality being under direct effect of the annual climatic cycle (reviewed in Goto *et al.*, 1999). This picture is consistent with the rapid spread of the species through a range of photic regimes spanning ~15° latitude on each of South and North America, from a reduced number of Palearctic colonisers (Balanyà *et al.*, 2006).

Concluding tips and future challenges

Gauging present data to historical data from different seasonal conditions can mislead inferences about global warming effects from biomonitors that cycle seasonally. Table 1 outlines basic

Table 1. Generalisations to keep in mind when planning/using updates of historical records to infer putative long-term effects of global warming from biomonitors that cycle (or are suspected to) with the seasons.

- Quality of the historical record
  - Avoid historical records lacking collection date information
  - Exact dates preferable to approximate/uncertain dates
  - Timing with respect to calendar date of the vernal equinox
  - Re-sampling over the growing season preferable to single isolated observations
  - Climatic time series data to aid anticipate shifts in season timing
- Potential effects of lengthening of the growing season
  - Vary across species and taxonomic groups
  - Increase with latitude/altitude
  - Increase at seasonal transitions, particularly at the spring-summer transition
  - Overestimates from non-photoperiodic organisms
  - Underestimates from organisms bounded by photoperiodism
  - Synergistic, antagonistic inputs from species interaction mismatches

tips to help prevent this potential drawback. Updating historical records consisting of repeated observations over the growing season should be superior to focusing on historical records consisting of isolated observations. Historical records lacking detailed dating information should be avoided. Ongoing monitoring programs should place special care on facilitating future tracking efforts by reporting all the required information, including the time of the day.

Retrospective synoptic climatic conditions can be estimated using multivariate methods. Updates can then be scheduled using forecasts from climatic time series. Comparison of historical with present monitor configurations at calendar and estimated seasonal dates should permit adjusting for season timing shifts. This two-point updating approach would help identifying situations in which responses are bounded by photoperiodism. Most animals and plants that have been tested use day length to switch between phenophases. Most examples of seasonal cycling of gene frequencies involve *Drosophila* allozyme and chromosomal polymorphisms. The *Drosophila* species in which most of these observations have been made, either are non-photoperiodic or do not exhibit a clean photoperiodic response. This lack of strong photoperiodism makes these *Drosophila* idiosyncratic among arthropods and many other vertebrate and invertebrate taxa. Day length at temperate and polar environments provides the most reliable anticipator cue of future seasonal conditions over evolutionary time (Bradshaw & Holzapfel, 2007). Organisms that lack the predictive power conferred by photoperiodism are therefore more likely forced to change genetically in response to direct effects of temperature (Bradshaw & Holzapfel, 2007, 2008). Because of this reason, together with short generation times and large population sizes, thermal adaptation to recent, rapid climate warming by an animal has been predicted to be demonstrated first in *Drosophila* (Bradshaw & Holzapfel, 2008). Species are reacting differently to climate change (Menéndez, 2007; Parmesan, 2007; Both *et al*., 2009). Eventually, deciding on the best time to update may require taking into account the responses of other species on which the focal species depend (Both *et al*., 2009). *Drosophila subobscura* is a trophic generalist, therefore it may be less sensitive to mismatches with its interacting partners, as it can more easily switch to alternative feeding substrates than specialist species.

Still, many biomonitors are not subject to the timing of re-sampling. For example, comparisons of previous (e.g., resurrected ancestors that have undergone a period of dormancy) and current (their descendants) populations reared under common-garden conditions in protists and microinvertebrates, mosquitoes, birds, and plants (Bradshaw *et al*., 2004; Coppack & Pulido, 2004; Angeler, 2007; Franks *et al*., 2007); differences in migration direction of hand-reared chicks (Pulido, 2007); pedigree analyses that already take year-to-year variation into account (Réale *et al*., 2003).

One might wish to know how populations and species respond genetically to global warming by (1) calendar date, (2) seasonal condition, and (3) with respect to photoperiod. None of these is any more important *a priori* than the others – they just have different meanings. Accurate estimation of long-term directional genetic responses to global warming from short-term seasonally cycling time series requires control for seasonal condition. This does not mean that resulting variates are causally unrelated to the annual climatic cycle. For example, the long-term directional shifts of some common chromosomal inversions of *D. subobscura* in northwest Iberia could be a cumulative effect of seasonal selection ensuing from the expansion of the warm season (e.g., $O_{\underline{3+4}}$, a southern inversion whose increase was mostly accounted for by the increase in spring maximum temperatures), in addition to climate warming direct effects (e.g., O_{ST}, a northern inversion whose decrease was mostly accounted for by the increase in summer minimum temperatures) (Rodríguez-Trelles & Rodríguez, 1998; see also Fig. 2). Significantly, this might represent the first record of a rapid genetic response to the global warming induced lengthening of the growing season.

The ‘season-sensing’ character set and its underlying genetics remain largely uncertain but must involve many loci that are fitness-costly to optimise simultaneously. It is not possible to anticipate how long into the future currently observed associations between genetic markers (e.g., chromosomal inversions) and seasonal/thermal and photoperiodic environments will persist. They may change as a result of depleted genetic variation, new mutations, or eventual trade-offs ensuing from traits not presently under selection (Pörtner *et al*., 2006; Reusch & Wood, 2007; Skelly *et al*., 2007; Gienapp *et al*., 2008). It is already apparent that at least some *Drosophila* inversion polymorphisms change correlatively with global warming. The challenge now is to grapple effectively with causation (Santos *et al*., 2005; Laayouni *et al*., 2007).

Acknowledgements

We are indebted to two anonymous reviewers for their valuable comments. MAR is supported by a CICYT grant (CGL2006-03000/BOS) from the Ministerio de Educación y Ciencia (Spain).

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Accepted 4 October 2009

Editor: Jacobus Boomsma
Associate editor: Joop Ouborg