Trophic generalism in the winter moth: a model species for phenological mismatch

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1. ABSTRACT

Climate change has the potential to disrupt phenological synchrony among interacting species that vary in their phenological sensitivity to temperature. The phenological synchrony observed between winter moth *Operophtera brumata* caterpillars and oak leafing in spring has become an emblematic test case of this phenomenon, with caterpillars seemingly advancing their phenology more than their host-plant. However, work on this trophic interaction—and on phenological mismatch more widely—routinely overlooks the potential for trophic generalism to buffer the negative effects of mismatch. In the largest study of its kind—using over 3500 individuals reared from egg to pupa—I tested the performance of winter moth caterpillars from four UK populations across nine host-plant species, and considered how adaptation to locally abundant host-plants may modulate performance in different populations. I found that caterpillars survive and grow well across a range of host-plant species, with some evidence of a host-plant by population interaction in performance. Contrary to widespread assumptions, oak seems a relatively poor host-plant species. Occupying a broad trophic niche may help consumers like the winter moth exploit a narrow phenological niche, whereby phenological variation among host-plant species buffers them against asynchrony with any one particular host-plant species. Determining the significance of trophic generalism in the ecology of consumers is a crucial first step towards assessing its role as a potential buffering mechanism and, hence, evaluating the true threat posed by mismatch.

2. INTRODUCTION

Over recent decades, rising global mean temperatures (IPCC, 2021) have brought about directional shifts in phenology across a range of taxa (Cohen et al., 2018; Roslin et al., 2021; Thackeray et al., 2016). For many organisms, survival or successful reproduction can depend on timing a stage in their life history such that it is synchronised with the timing of another species. For example, temperate fish species that fail to spawn at the correct time can find their offspring mismatched with the maximum abundance of a food supply, with negative consequences for recruitment to the population (Cushing, 1969, 1990). Similarly, birds that mistime their breeding relative to the maximum availability of an ephemeral insect food supply can suffer reductions in offspring condition (Samplonius et al., 2016), individual fitness (Reed et al., 2013), and perhaps even population size (Both et al., 2006; Mclean et al., 2016; though cf. Samplonius et al*.,* 2020). There is a growing concern among ecologists that divergent phenological responses to changing global temperatures in different species could lead to a temporal decoupling of many such timed interactions—'phenological asynchrony'—with potentially catastrophic effects on populations and perhaps whole ecosystems (reviewed in Samplonius et al*.,* 2020; Iler et al*.,* 2021).

In a trophic context, phenological asynchrony is most likely to occur where a consumer exploits a single, ephemeral resource—this makes precise timing particularly important, and in theory even small temporal misalignments can lead to reductions in fitness (Cushing, 1967, 1969, 1990; Durant et al., 2007; Hjort, 1914). Although most prior research focusses on simplified food-chains (see Samplonius et al. 2020; Weir 2022), the complex structure of food-webs in nature could reduce dependence on any one resource. Samplonius et al. (2020) found that in 74% of studies of phenological mismatch both the consumer's dependence on a resource and the ephemerality of that resource were assumed *a priori* and not directly tested. Very few studies explicitly test the extent to which consumers are generalists (Samplonius et al., 2020) and therefore the potential for generalism to buffer the negative impacts of asynchrony with any one trophic resource.

A plurality of studies which investigate the effects of phenological mismatch use the spring woodland tree/caterpillar/bird food-chain as a model system (Samplonius et al., 2020). Due to its abundance, the caterpillars of the winter moth *Operophtera brumata* (L.) are often taken as representative of the primary consumer level in this chain and it has become a standard study organism for phenological research (Charmantier et al., 2008; Cole et al., 2015, 2021; Hinks et al., 2015; Shutt et al., 2019; Visser et al., 2021). Winter moth caterpillars hatch as foliage appears on trees in early spring (Skinner, 2009), ready to exploit this newly available food resource. Over the last few decades, evidence has accumulated suggesting that the fitness of winter moth caterpillars depends to a very large extent on precisely matching their phenology with that of their host-plants (van Asch and Visser, 2007; Buse et al., 1999; Van Dis et al., 2023; Van Dongen, 2006; Feeny, 1970; Kerslake and Hartley, 1997; Tikkanen et al., 1998; Tikkanen and Julkunen-Tiitto, 2003; Tikkanen and Lyytikäinen-Saarenmaa, 2002; Wint, 1983). Caterpillars which hatch too early find themselves with no foliage to feed on and starve (Wint, 1983); those hatching later are forced to feed on more mature foliage which has undergone structural changes and accumulated secondary chemicals reducing its nutritional value, and hence its (Feeny, 1968; Feeny, 1970). The result is strong stabilising selection for close synchrony between the timing of caterpillar egg hatch and the timing of bud-burst on their host-plants (van Asch et al., 2007; Van Dis et al., 2023; Tikkanen and Julkunen-Tiitto, 2003). But framing the winter moth as reliant on synchrony with a single host-plant species (Table S1) may misrepresent its diet—in nature, we find a complex food web of many different interacting caterpillar and host-plant species.

Temperate spring-feeding caterpillars as a group are typically trophic generalists (Henwood et al., 2020; Maitland Emmet and Heath, 1992; Porter, 2010). Some degree of polyphagy in the winter moth has been observed historically (Allan, 1979; Henwood et al., 2020; Maitland Emmet and Heath, 1992; Meyrick, 1895; Porter, 2010; Stainton, 1859; Stokoe, 1948; Waring et al., 2017), with caterpillars recorded feeding on plants from 31 different genera across 15 families (Robinson et al., 2010). Occupying a broad niche can be optimal in uncertain environments (Levins, 1968) and so, faced with uncertainty in various aspects of the environment (e.g. unpredictability of the developmental stage of any available leaves at the onset of spring) a generalist diet might have arisen in this species as a buffer against being mistimed with any one particular host-plant individual or species.

However, trophic generalism in the winter moth is largely only mentioned in passing in the phenological literature (Table S1). Instead, the focus has mainly been on a single host-plant, English oak *Quercus robur* L. (Table S2; Roland and Myers, 1987; Buse et al., 1998, 1999; Tikkanen and Lyytikäinen-Saarenmaa, 2002; Tikkanen and Julkunen-Tiitto, 2003; Van Dongen, 2006; van Asch et al., 2007; Mannai et al., 2017; Kulfan et al., 2018). The reasons for this emphasis seem to be largely historical, tracing back to the earliest considerations of phenological synchrony in the winter moth (e.g. Thomson, 1954; Feeny, 1968; Varley et al., 1974). The focus on oak creates the impression of winter moth populations facing an ephemeral and moving resource peak in spring (young, nutritious oak foliage), as the oak itself responds plastically to temperature (Roberts et al., 2015). On the other hand, feeding on a wide range of host-plant species that vary in their leafing phenology may extend the period over which young leaves are available overall, serving as a buffer on trophic mismatch.

Despite the extensive literature on the winter moth, even the relative importance of very widespread and abundant alternative host-plant species, such as birch *Betula* spp., has rarely been considered (Table S2). Furthermore, even though there is considerable variation and turnover in flora throughout the Holarctic distribution of the winter moth, previous studies considering local adaptation to host-plant are very limited in geographical scale (e.g. Kerslake and Hartley, 1997; Tikkanen et al., 2000). Since the flightless female winter moths exercise very little (if any) taxonomic discrimination with regard to the host-plant that their offspring will find themselves on, a broad diet could give this species flexibility, with local adaptation potentially fine-tuning and optimising performance at a local level.

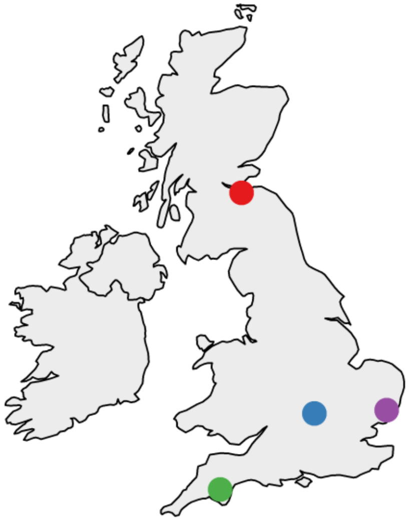
To test whether there is potential for trophic generalism to serve as a buffer against phenological mismatch with host bud-burst, I quantified the performance of winter moth caterpillars across a range of common and widespread host-plant species. Understanding the performance effects of different diets is a vital first step in assessing the capacity for alternative host-plants to act as buffers against mismatch. (**Aim A**) I conducted an extensive assay of performance across several metrics on nine host-plant species, using 3600 individual caterpillars—the largest such experiment to date. (**Aim B**) Additionally, to test for significant geographical divergence in performance across host-plant species (consistent with local adaptation), I assayed livestock sampled from four populations across Great Britain. I found that although performance varied substantially, a wide range of host-plants could be utilised effectively. In contrast to expectations, oak proved a relatively poor host-plant, in terms of caterpillar performance. I consider the implications of these results for the resilience of temperate woodland food-webs under climate change (of which the winter moth forms a crucial part), and for the impacts of phenological asynchrony on trophic generalist versus specialist taxa more broadly.

3. MATERIALS AND METHODS

3.1. Source populations

I obtained winter moth ova from four populations across Great Britain (Table 1). I collected females from the Edinburgh population using trunk traps, modelled on those described by Varley et al. (1974). I collected a total of 165 females between 25 Nov 2019 and 8 Jan 2020, across 72 traps.

Table 1. UK collection sites of winter moth livestock used in the host-plant assay, with a description of the local flora.



	Site	Co-ordinates	Habitat Characteristics (Alt.)
🔵	BUCKINGHAMSHIRE Hill Farm Cottage, Buckingham (VC 24)	51.978946°N -0.983623°E	Hamlet surrounded by grazing pasture. Small garden orchard of apple, pear, plum, cherry, fig and apricot. Dry area, with extensive hedges of hawthorn, maple, ash, blackthorn. 110m
🟢	DEVON Dart Valley Nature Reserve, Poundsgate (VC 3)	50.530946°N -3.849855°E	Ancient, damp, primarily oak woodland, situated in heathland. 280m
🔴	EDINBURGH Hermitage of Braid LNR, Edinburgh (VC 83)	55.919501°N -3.197014°E	Exposed patch of mature oaks on the edge of a large mixed woodland, adjacent to grassland. Sycamore abundant throughout. 105m
🟣	SUFFOLK Ipswich Golf Course, Ipswich (VC 25)	52.042964°N 1.215717°E	Sheltered site at the edge of a mixed woodland of oak, birch, sycamore, Scots pine. Surrounded by dry heathland and short-cropped grassland. 20m

Entomologists located near the three other sites each provided me with a minimum of fifteen female winter moths from each (see Table 1). Individuals from these populations were collected manually by searching trunks after dark by torchlight. These populations were selected due to their geographical spread and variation in local habitat types.

3.2. Rearing methodology

After collection, females were placed individually in 75 x 25mm glass tubes with a wad of cotton at the bottom to act as an egg-laying medium. Females from all sites were stored at approx. 5°C in complete darkness and allowed to lay freely. Approximately one month later, all tubes were examined and the dead females were removed. Ova from a total of 126 females from the Edinburgh site, 15 from Buckinghamshire, 14 from Devon, and 19 from Suffolk were obtained.

When foliage became available in spring, ova were removed from cold storage and placed at room temperature (approx. 20°C) to stimulate egg hatching. A subset of ova, sampled from across all broods, were removed concurrently and allowed to hatch. Exposure to relatively high temperatures helped ensure individuals hatched at the same time, despite inter- and intra-brood variation in the temperature requirements for eclosion. Caterpillars were assigned at random from each brood to each treatment group, and subsequently to each rearing culture within that treatment group.

Caterpillars were reared in mixed-brood groups of twenty individuals (a “culture”), firstly in small 75 x 50 x 15mm transparent plastic containers and then, at around the third instar, in larger 500ml disposable plastic containers (Fig. 1 and S1; for a discussion of the mixed-brood culture rearing method see Appendix 2). Cultures were established concurrently from caterpillars hatched in the previous 24hrs. The rearing containers were lined with white absorbent paper towels. Freshly excised food was placed in each container and examined daily to check its condition and how much remained. Typically it was replaced daily, no less than every second day (Fig. 1). Caterpillars were provided with an excess of plant material at all times such that the quantity of food was never a limiting factor to growth. The tissue lining of the container was replaced each time new food was provided. Caterpillar rearing cultures were maintained together at room temperature (approx. 20°C) with a 10:14 light:dark regime.

At the completion of their development the caterpillars pupated in the tissue at the base of the container. After all larvae had pupated, excess host-plant material was removed and the containers were stored at room temperature. One month after pupation, pupae were removed, laid out on cotton for emergence in sealed plastic container, and stored outdoors under a canopy at ambient environmental temperature (Stirlingshire; 56.069°N, -3.767°E).

3.3. Host-plant assays

The aims of this experiment were to determine:

(**Aim A**) how caterpillar performance differed among nine common host-plant species;

and

(**Aim B**) if performance differed across the different geographical populations in a manner consistent with local adaptation.

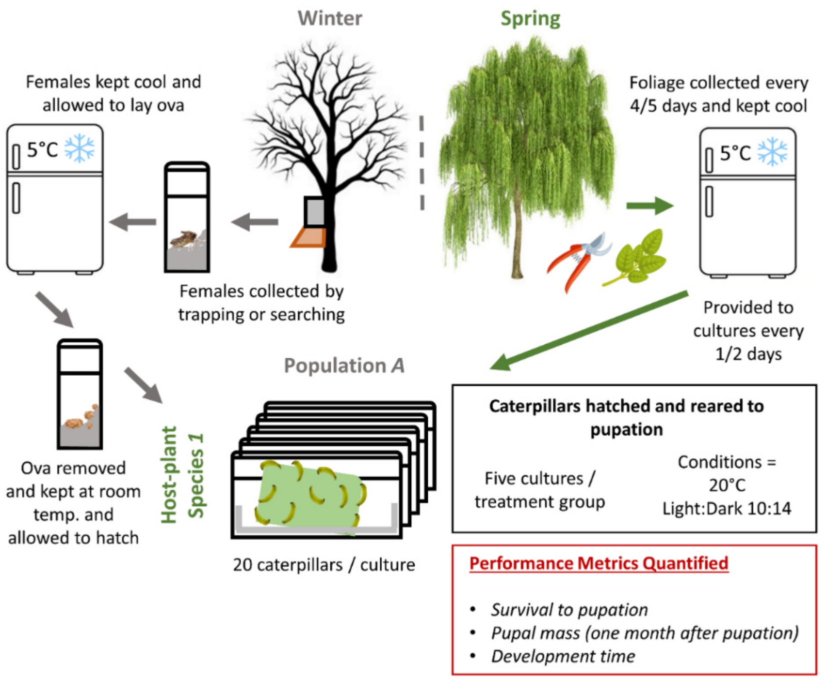


Figure 1. Schematic of the experimental design. Winter moth livestock from four British populations were reared to pupation on foliage from nine different host-plant species. Caterpillars were reared in cultures of 20 individuals, assigned to each culture randomly across broods. Performance in each host by population treatment was quantified on three metrics: survival, pupal mass attained, and development time (time from egg hatch to pupation).

Nine known host-plant species of the winter moth (Robinson et al., 2010) which are common and widespread in Britain were selected for use in the assays: alder *Alnus glutinosa*, apple *Malus domestica*, birch *Betula pendula*, cherry *Prunus avium*, hawthorn *Crataegus monogyna*, oak *Quercus robur*, sallow *Salix caprea*, sycamore *Acer pseudoplatanus*, and willow *Salix alba* (Fig. S2). One hundred larvae from each source population were randomly assigned from across broods to each of the nine host-plant species, at twenty larvae per rearing culture (Fig. 1), totalling 3600 caterpillars. Since it seems likely that caterpillar fitness will depend on foliage age (van Asch et al., 2007; Olli-Pekka Tikkanen and Julkunen-Tiitto, 2003), the calendar start date of each host-plant treatment was staggered, such that for each host-plant species the experiment began when a sufficient number of individual trees could be sampled in the field at the appropriate phenological stage (defined as where buds were opening and leaf shape was becoming recognisable; Table S3). Fresh foliage was collected from a dozen individual trees of each species every four to five days as cut sprigs 15cm long and stored in airtight plastic bags at approx. 5°C in a tabletop refrigerator (Russell Hobbs RHCLRF17) until required for feeding. Foliage was collected from trees near Falkirk (Stirlingshire; 56.069°N, -3.767°E) and Kincardine (Fife; 56.057°N, -3.613°E).

In order to minimise the effects of individual variation in leaf properties *within a host species* (see for example Laitinen et al. 2005; Lindroth 2012; Kos et al. 2015), leaves from across all the sampled tree individuals were randomly assigned to each rearing culture, such that larvae always had access to foliage from a range of different host-plant individuals belonging to the same species.

The performance of caterpillars in each treatment group was quantified by measuring:

- the survival of each individual from hatch to pupation;
- the final pupal mass attained by each individual one month after pupation (measured using a Mettler AJ50 balance, to 0.0001g);
- and, the time taken for caterpillar development from egg hatch to pupation (Fig. 1).

Since larvae were reared in groups it was not possible to relate each of these values to a specific individual.

3.4. Statistical analyses

Analyses of larval performance were conducted in *R* v.4.0.3 using MCMCglmm (Hadfield, 2010). Survival to pupation ("Surv", binomial response with binary outcome), pupal mass ("Mass", Gaussian), and development time ("Dev Time", Gaussian) were each modelled separately (Table S4). Models included the following random terms:

Host-plant species, to allow estimation of the overall differences in performance among host-plants across all populations (Aim A).

Population, to allow estimation of differences in the average performance of different populations across all host-plants. If performance varied significantly by population, this may suggest systematic problems with the experimental design, e.g. livestock from one area experiencing different conditions.

Host-plant species : Population, to allow estimation of population-specific differences in performance on different host-plants (Aim B), and examination of whether geographical divergence in performance between populations is consistent with local adaptation.

In each model Rearing Culture was fitted to control for differences between each culture. In the Mass model, sex was included as an additional random effect because mass varies by sex but could only be determined by sexing the pupae, not larvae, and it could therefore not be included in other models.

Because individual caterpillars were reared in mixed-brood cultures, it was not possible for most models to include a random effect of brood. To assess the potential of this unaccounted-for source of pseudoreplication to bias inferences I conducted extensive simulations, manipulating levels of within- and among-brood variance and assessing the impact on model estimates (bias and precision) and on false positive rates and power. The simulations suggest that the experimental design generates conservative estimates for the variance and significance of the focal model parameters, and is therefore robust in terms of addressing the stated aims of this study (see Appendix 2).

All models were run for 1500000 iterations with a 500000 burn-in and thinning every 100 iterations. In the binomial model for survival, default priors were used for the fixed effects (mean = 0, with a large variance), inverse-Wishart priors for the random effects, and the residual variance was fixed. In the remaining Gaussian models, the default priors were used throughout.

Variance components were estimated on the link scale for each model. Using the posterior distributions of survival, pupal mass, and development time, I also estimated the rate of development (mg/day) and projected absolute individual fitness (eggs/female) in each treatment group (for detailed explanation, methodology, and derivation, see Appendix 1).

3.5. Detecting local adaptation

Two different criteria have been advanced for detecting local adaptation: (1) that a local genotype performs better than any other genotypes in that local environment ("local vs foreign"); and (2) that a local genotype performs better in its local environment than in foreign environments ("home vs away"). Where a genotype is locally adapted, both of these criteria will often be fulfilled. However, Criterion 2 is likely to be misleading where there are significant underlying differences in average fitness in different environments, and it should not be regarded as a definitive test (Kawecki and

Ebert, 2004). In the common garden setup of this experiment the 'environments' are alternative host-plants. Comparison of the results of the model outputs and the characterisations of the local flora of each population (Table 1) allow for an evaluation of these criteria in this study. For example, we would expect a locally adapted genotype derived from a predominantly oak woodland site to show relatively higher fitness on oak than genotypes derived from other populations where oak is less abundant, under Criterion 1.

4. RESULTS

4.1. (Aim A) Caterpillar performance and fitness across host-plant species

Survival

The mean individual probability of survival to pupation across all treatment groups and populations was 0.18. Survival probability varied significantly among host-plant species (Table 2; Fig. 2; 47.20% of variance on the link scale, 95% CIs: 19.07, 78.59). Relative to oak (0.16, CIs: 0.06, 0.27), survival was lower on alder (0.03, CIs: 0.00, 0.06) and hawthorn (0.04, CIs: 0.00, 0.07) and markedly higher on willow (0.47, CIs: 0.30, 0.65), but did not differ significantly between oak and the remaining host species (Fig. 3).

Pupal mass

Mean pupal mass across all treatment groups one month after pupation was 26.17mg (sd = 7.55). Female pupae (26.80mg, sd = 8.10) were slightly heavier on average than those of males (25.6mg, sd = 7.01). Pupal mass varied substantially among host-plant species (Table 2; Fig. 2; 42.33% of variance, CIs: 12.19, 78.19). Pupal mass was significantly higher on all other host-plant species than on oak (Fig. 3). Host-plant species fall into three discrete groups with regard to pupal mass attained, with apple and cherry being intermediate between oak and all the remaining species (Fig. 3).

Development time

Mean development time across all treatment groups was 32.14 days (sd = 6.54). Development time varied substantially across host-plant species (Table 2; Fig. 2; 45.73% of variance, CIs: 8.94, 79.00). Development time on oak did not differ significantly from the mean, but it was significantly shorter than on some other host-plants, such as hawthorn, apple, and cherry (Fig. 3).

Estimated rate of development

Mean rate of development across all treatment groups was 0.76 mg/day (CIs: 0.50, 1.09). On a majority of host-plant species, the estimated rate of development does not depart significantly from the mean (Fig. 3). However, rates were significantly higher than average on willow 1.03 mg/day (CIs: 0.78, 1.34) and lower on apple (0.56, CIs: 0.43, 0.72) and cherry (0.52, CIs: 0.41, 0.66). Notably, development rate on oak (0.74, CIs: 0.44, 1.11) did not differ significantly from any of the other host-plant species (Fig. 3).

Fitness

The arithmetic mean of estimated fitness (projected eggs per female per treatment, see Appendix 1) across all treatment groups was 22.19 (sd = 31.80). Fitness was significantly higher than average on birch (44.87, CIs: 16.65, 81.85) and willow (94.29, CIs: 45.62, 144.89), and lower on oak (4.66, CIs: 0.46, 11.59), alder (2.20, CIs: 0.29, 5.99), and hawthorn (2.66, CIs: 0.38, 6.82). Relative to oak, fitness was higher on birch and willow—two abundant and widespread species (Fig. 3).

4.2. (Aim B) Geographical divergence in caterpillar performance

Source population main effects explain a sizeable portion of variation in development time (Table 2; Fig. 2; 35.14%, CIs: 3.17, 77.49), which is generally more prolonged in livestock sourced from the Devon and Suffolk populations (Fig. 4 and S3). Although development time is generally more protracted in these two populations, between-population differences in rate of development are less obvious and less pronounced (Fig. 4). For the remaining response variables, the source population variance posterior means and upper credible intervals were quite small.

The host by population interaction term explained a substantial quantity of variation in survival (24.08%, CIs: 6.41, 43.71), pupal mass (13.62%, CIs: 0.00, 25.48), and development time (9.08%, CIs: 0.57, 19.15) (Table 2; Fig. 2). This indicates differences in performance on the same host-plant species among source populations which may be a result of genetic divergence and local adaptation. However, although performance on a given host-plant varied considerably among populations, there was no obvious indication of local adaptation when comparing performance with the flora of each site (Table 1).

5. DISCUSSION

In this large-scale experimental study of performance, assaying 3600 caterpillars across nine host-plant species, I found that winter moth caterpillars are highly polyphagous trophic generalists. Although performance varied across host-plants, caterpillars survived and grew well on a wide range of species. There were clear differences between populations in terms of the performance on different host-plants, although this did not obviously correlate closely with the local flora of each site. Oak proved to be an unexceptional host-plant, running counter to the common framing of English oak (*Q. robur*) as the most significant host-plant species for the caterpillars of this moth in the wild (Table S1) and its use as the model host-plant in studies of phenological asynchrony (Table S2). Indeed, variation in performance among hosts, as shown here, may imply substantial variation in the fitness effects of asynchrony across host-plant species. These results present a crucial first step in evaluating the role of trophic generalism as a potential buffer against phenological mismatch. In the case of the winter moth, the evidence presented here is consistent with the hypothesis that a broad diet might confer resilience in the face of asynchrony induced by climate change. The clear next step in order to verify this hypothesis is to conduct direct tests in a natural setting.

5.1. The significance of oak as a host-plant of the winter moth

If oak is at all notable as a host-plant in the results presented here, it is as a relatively poor one. First, I found that developmental time—a well-established, though inconsistent, signifier of environmental stress in Lepidoptera (Awmack and Leather, 2002; Goulson and Cory, 1995; York and Oberhauser, 2002)—is considerably shorter on oak than on all other host-plant species, and no compensatory effect of rate of development was observed (Figs. 3 and 4). Second, pupal mass is significantly lower on oak (Fig. 3), consequently producing females with a greatly reduced fecundity (Appendix 1). Mean estimated fitness lags far behind many species, at the third lowest level attained in this

Table 2. Summaries for survival, pupa mass, and development time models.

	Coefficient/Variance (Mean and CIs)	**Effective Sample Size**
SURVIVAL MODEL		
Fixed Terms		
Intercept	-2.296 (-3.351, -1.304)	10000
Random Terms		
Rearing culture	0.000473 (0, 0.00248)	1367
Population	0.04603 (0, 0.09103)	10000
Host-plant	2.096 (0.2705, 5.152)	9457
Population:Host-plant	0.8721 (0.3338, 1.507)	4341
MASS MODEL		
Fixed Terms		
Intercept	26.33 (19.57, 31.47)	10000
Random Terms		
Rearing culture	0.009934 (0, 0.03498)	9041
Population	0.6829 (0, 0.9573)	10000
Host-plant	33.35 (5.025, 79.51)	10000
Population:Host-plant	9.289 (3.708, 16.36)	2467
Sex	1695 (0, 77.49)	10000
DEV TIME MODEL		
Fixed Terms		
Intercept	33.94 (24.61, 42.27)	10000
Random Terms		
Rearing culture	0.007574 (0, 0.02736)	5620
Population	68.74 (1.433, 186.9)	1210
Host-plant	50.82 (10.19, 116.9)	10000
Population:Host-plant	8.624 (3.198, 15.08)	10000

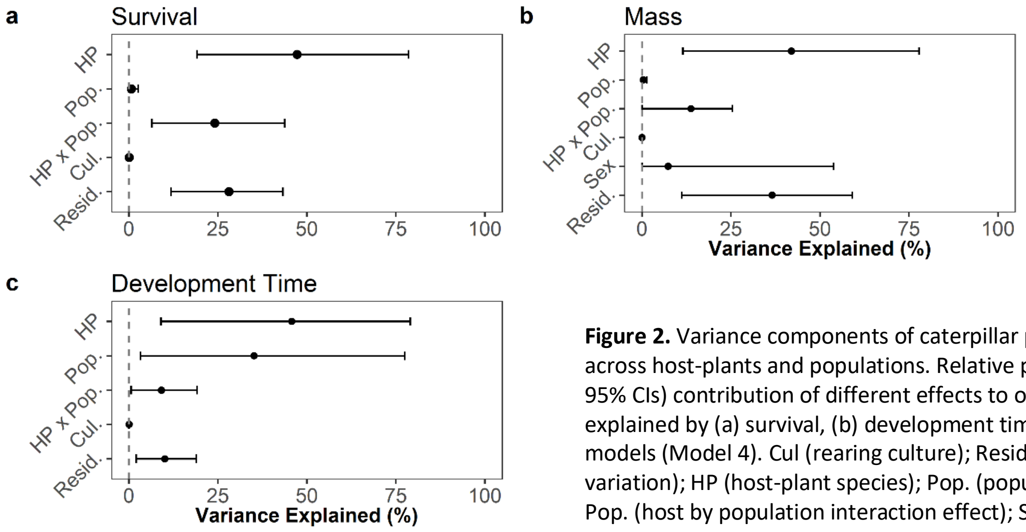


Figure 2. Variance components of caterpillar performance across host-plants and populations. Relative percentage (+/- 95% CIs) contribution of different effects to overall variance explained by (a) survival, (b) development time, and (c) mass models (Model 4). Cul (rearing culture); Resid. (residual variation); HP (host-plant species); Pop. (population); HP x Pop. (host by population interaction effect); Sex (individual sex). Estimates shown on the link scale.

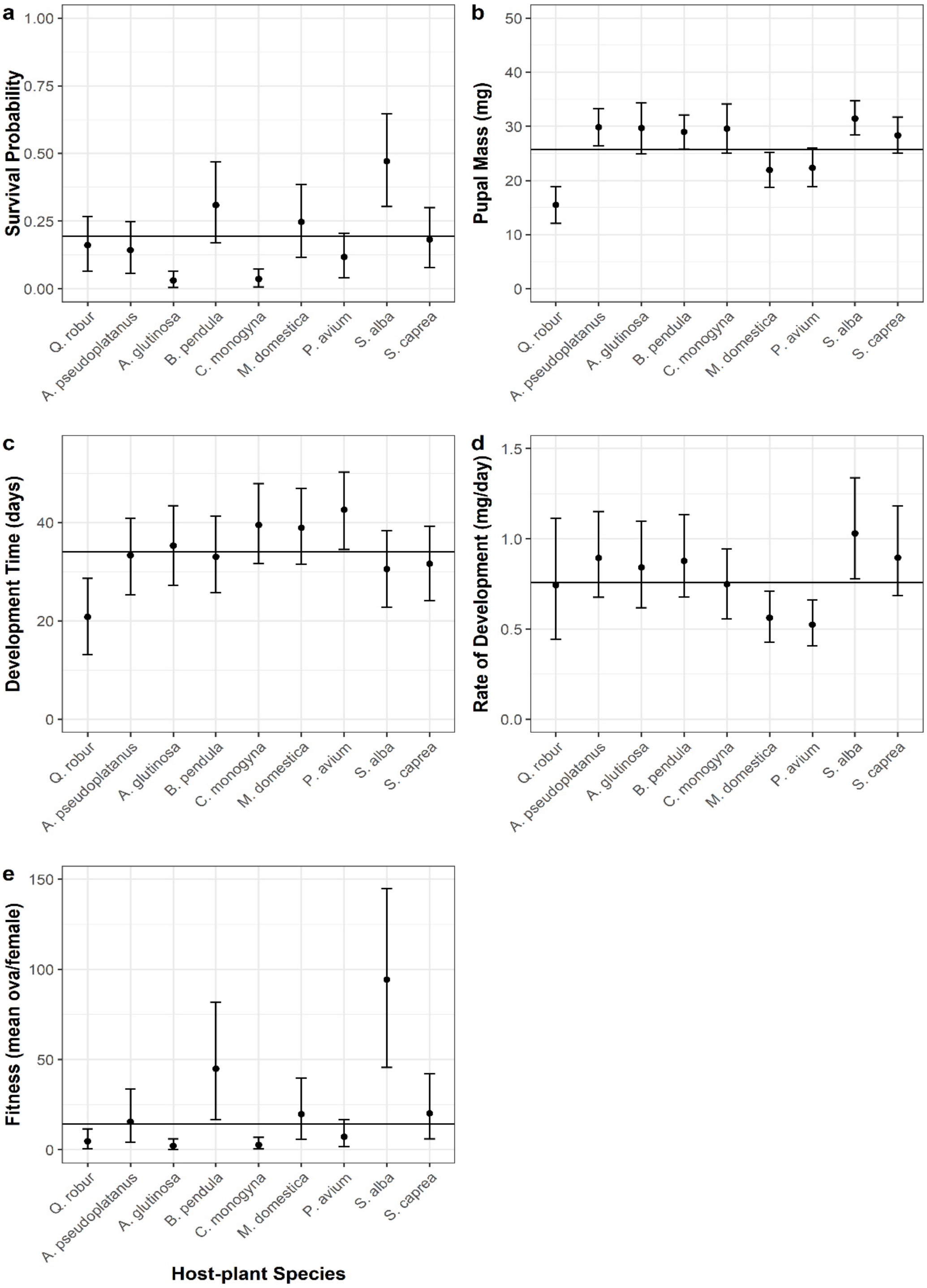


Figure 3. Overall winter moth performance on different host-plants, across all sampled populations. Performance quantified as (a) survival probability, (b) pupal mass, (c) development time, (d) rate of development, and (e) estimated fitness. Mean estimates and 95% credible intervals shown. Global mean for each performance metric shown by solid line. Vernacular names of host-plants are: alder *Alnus glutinosa*, apple *Malus domestica*, birch *Betula pendula*, cherry *Prunus avium*, hawthorn *Crataegus monogyna*, oak *Quercus robur*, sallow *Salix caprea*, sycamore *Acer pseudoplatanus*, and willow *Salix alba*.

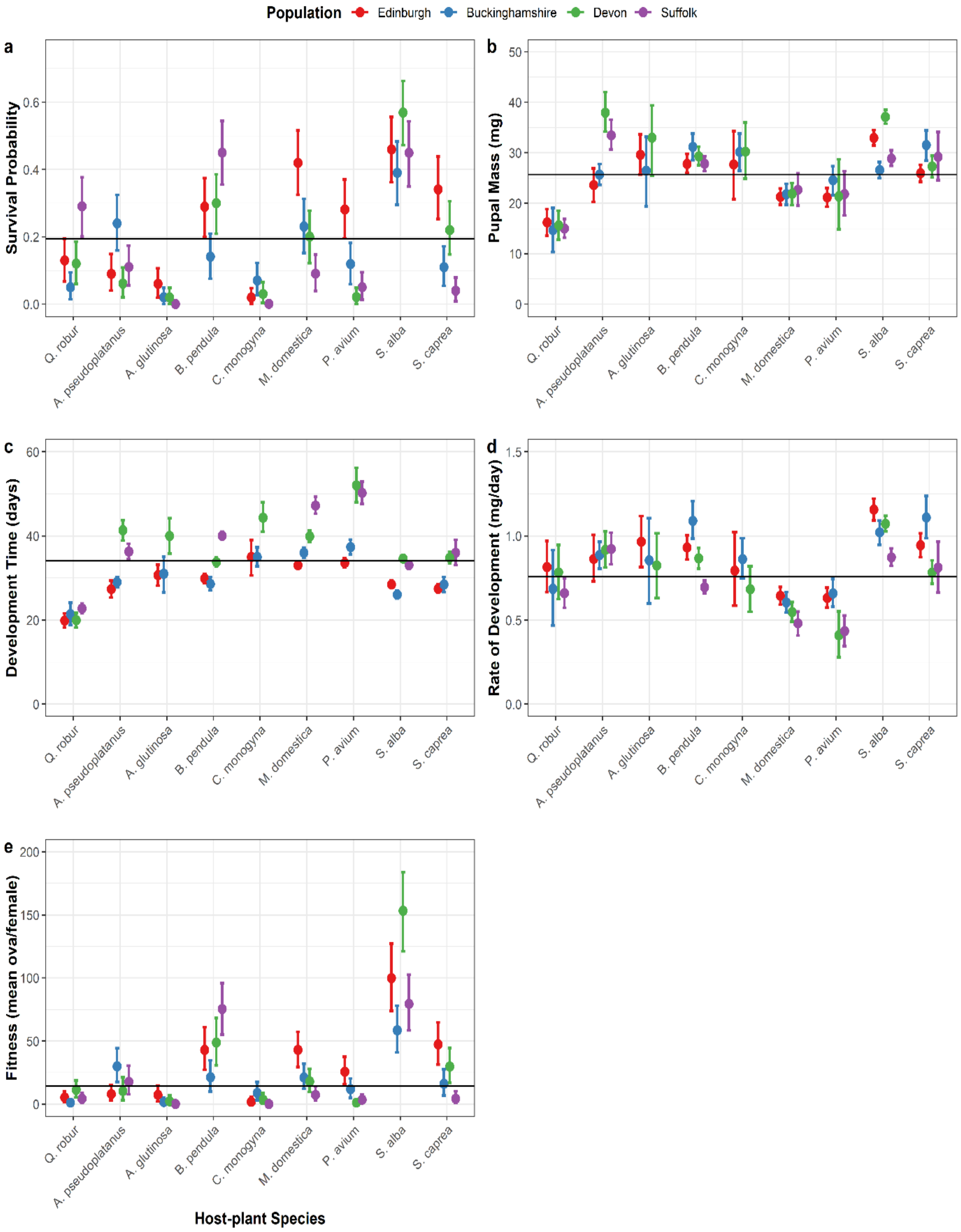


Figure 4. Winter moth performance in each host-plant by population treatment group. Performance quantified as (a) survival probability, (b) pupal mass, (c) development time, (d) rate of development, and (e) estimated fitness. Mean estimates and 95% credible intervals shown. Global mean for each performance metric shown by solid line. Vernacular names of host-plants are: alder *Alnus glutinosa*, apple *Malus domestica*, birch *Betula pendula*, cherry *Prunus avium*, hawthorn *Crataegus monogyna*, oak *Quercus robur*, sallow *Salix caprea*, sycamore *Acer pseudoplatanus*, and willow *Salix alba.*

experiment (Fig. 3). Indeed, fitness is markedly higher on some of the other common, widespread host-plant species, such as birch (four times higher) and willow (eight times higher).

The limited experimental work conducted prior to this study reports mixed results with respect to winter moth caterpillar performance across host-plants (Table S2). In some cases performance is indeed highest on oak (O'Donnell et al., 2019; Vanbergen et al., 2003), and even on evolutionarily novel oak *Quercus* species (e.g. N. American *Q. rubra*; Embree 1965, 1970). In other instances alternative host-plant species prove equally suitable or better—however, in a plurality of multi-species studies, oak results in average or mixed caterpillar performance, across a range of metrics (Cuming, 1961; Kirsten and Topp, 1991; Tikkanen et al., 2000; Tikkanen and Lyytikäinen-Saarenmaa, 2002; Wint, 1983). But performance has been found to vary substantially even in comparisons between oak species (Kulfan et al., 2018; Mannai et al., 2017; Wesołowski and Rowiński, 2008). In the classic study by Wint (1983), which is often cited as evidence for the importance of oak, the picture was also mixed (pupal mass was highest on oak, but survival was higher on almost all other host-plant species assayed). However, in these studies performance across hosts is often inferred or quantified incidentally to the main aim of the study, employing considerably smaller sample sizes, fewer host species, and limited geographical comparisons. Here, I have presented firm evidence of the extent—and potential ecological consequence—of trophic generalism in this species.

Lab rearing assays capture one aspect of dietary ecology: the palatability of the host-plant. In nature, different tree species and individuals at different stages of growth provide structurally different habitats, which might affect predation risk from vertebrates, parasitism, and susceptibility to adverse abiotic conditions. It would certainly be possible for extrinsic, non-dietary factors to differ sufficiently between host-plant species in the field *such that performance in the lab ran counter to abundance observed in nature*. But field studies comparing the abundance of winter moth caterpillars or defoliation across different host-plant species also find mixed results, with abundance being highest to mid-level on oak (see Table S2 and also: Shutt et al., 2019; Macphie et al., 2020). O'Donnell et al. (2019) found that abundance in the field and performance in captive rearing experiments ($N = 60$ / host-plant) were higher on oak than on other host-plants, but the extent to which abundance was greater on oak in the field *exceeded* the differences in lab performance. We are presented, then, with a complex array of interacting factors: for example, the reduced development time that we see in oak (Fig. 3 and 4) might also reduce exposure to predation, potentially compensating for any loss in mass and/or fecundity. Clarifying the paradox of these contradictions between lab and field studies, as well as directly quantifying the fitness effects of asynchrony across different host-plant species, are obvious directions for future work.

5.2. Evidence of local adaptation to floral composition

A winter moth caterpillar hatching in spring faces two principal uncertainties: the species and the phenological stage of its host tree. Variation in either of these factors can significantly impact overall performance (van Asch et al., 2007; van Asch and Visser, 2007; Feeny, 1970, 1976; Tikkanen and Julkunen-Tiitto, 2003; Wint, 1983). Given the limited dispersal ability of females and that they seem unable to exert much, if any, host choice (though see Connell, 2013), we might expect populations to adapt to locally abundant host-plant species. Although I find quite clear evidence of divergence between the British winter moth populations studied in this experiment, these differences do not obviously conform with predictions we might make based on the character of the flora at each collection site (cf. Table 1 and Fig. 4).

The scant data already available does seem to suggest that under at least some circumstances winter moth caterpillars perform better on locally prevalent host-plants. For example: on birch *Betula* spp. in Scandinavia (Belsing, 2015; Lavola et al., 1998); on bird cherry *Prunus padus* in Karelia (Tikkanen et al., 2000; Tikkanen and Lyytikäinen-Saarenmaa, 2002); and on heather *Calluna* from heathland populations (Kerslake and Hartley, 1997). Taken together with my data, this perhaps suggests that local adaptation to host-plant availability in this species occurs at a larger spatial scale than I considered here, or in other situations of extreme population isolation (e.g. islands, see Kerslake & Hartley, 1997). Determining the scale at which winter moth populations may be adapted to local flora would necessitate further investigation with higher levels of population replication.

In nature, relative phenological synchronisation between caterpillars and their host-plants may be a more significant determinant of overall fitness than the inherent palatability of a host-plant (van Asch et al., 2007; Tikkanen and Julkunen-Tiitto, 2003)—the most abundant host may not necessarily be the best host. If the performance effects of asynchrony vary among host-plants, then selection favouring feeding on the host-plant on which the effects of asynchrony are least severe (or the rewards of synchrony are greatest) may outweigh pressure to optimise performance on the most abundant host-plant species.

5.3. Polyphagy as buffering in an uncertain environment

By hatching in early spring and exploiting the young foliage of their host-plants, winter moth caterpillars occupy a narrow *phenological niche.* This is driven by selective pressures arising from variation in host palatability with time—the cost of asynchrony (van Asch et al., 2007; Tikkanen and Julkunen-Tiitto, 2003). Trophic generalism may be one mechanism by which they persist in their complex, heterogeneous ecological environment—consisting of many host-plant species, each varying in leafing phenology, unevenly spatially distributed and unequally palatable. By decreasing specificity in one aspect of their niche, winter moth caterpillars are able to specialise on a narrow phenological niche. Although trophic generalism is maintained throughout the distribution of the winter moth, we see indications that performance on particular host-plant species can be modulated in certain environments and populations, perhaps increasing fitness on locally abundant hosts while still being able to persist on many. Although trophic generalism prohibits specialisation on one host-plant, it likely results in a higher geometric mean fitness over time (Childs et al., 2010; Dempster, 1955) because of the substantial fitness costs associated with asynchrony (Weir, 2022).

The evidence I have presented here suggests that throughout its range there are many plant species acceptable to winter moth caterpillars, on which performance is at least comparable to oak. This ability to effectively utilise a very large range of host-plant species might act as a diversified bet-hedging strategy and ameliorate the negative effects of asynchrony with bud-burst on any one host-plant species (Weir, 2022). In order to assess this potential mechanism for buffering mismatch, future studies should seek to directly test its operation in nature. An obvious testable prediction might be that in years of high mean asynchrony, winter moth populations should perform better in mixed versus low diversity woodlands, or should experience shallower inter-annual fluctuations in population size. The results of such experiments would be of far-reaching applicability and interest, beyond the narrow context of this one consumer species, because these principles are likely to generalise very widely.

The inherent resilience of the winter moth to a temporally uncertain niche and asynchrony with any one particular host-plant—as evinced by its success and abundance—might help buffer their populations against future climatic changes affecting phenology, and contribute to the stability of the wider ecosystem of which this species forms a crucial part. Beyond the winter moth, trophic generalism in consumers has the potential to buffer mismatch in a range of phenologically synchronised systems. In insectivorous birds—another heavily researched system—we still have a relatively incomplete understanding of how variation in dietary composition can affect fitness and how these effects vary across time (Macphie, 2022). The example of the winter moth challenges us to revaluate the idea that phenological asynchrony is uniformly and severely negative for fitness. It serves to illustrate how critically important it is that we consider the wider ecological context of a species before we can expect to make robust projections as to the effects of climate change on their populations, or on those of species with which they interact.

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SUPPORTING FIGURES/TABLES

Table S1. The role of oak in the life of the winter moth: a selection of the phenological literature. There is a general consensus among widely cited studies that English oak *Quercus robur* is the most important (and preferred) host-plant of the winter moth in the field. Number of citations shown underlined after reference, obtained from Google Scholar (accessed 3 February 2023), provide a rough estimate of the influence of each publication on the field.

Views on the role of oak *Quercus robur* as a host-plant
"For example, winter moth (*Operophtera brumata* L., Geometridae) larvae develop into heavier pupae when fed on young oak leaves than when reared on hazel (*Corylus avellana* L.) or blackthorn (*Prunus spinosa* L.), two species commonly used as host plants in the field (G. R. Gradwell, personal communication)" **Feeny (1970)**, 2404
"Larvae of the winter moth are able to feed on a wide range of trees and shrubs, but they are especially abundant on oaks (*Quercus robur*), which they sometimes defoliate." **Varley *et al.* (1974)**, 1101
"Most of these losses are related to the degree of asynchrony between larval eclosion and the bud burst of the primary food-plant (*Quercus robur*)." **Wint (1983)**, 143
"The winter moth *Operophtera brumata* is one of the most common forest insects in Central Europe. The larvae feed on the oak, *Quercus robur*, as well as on several other species of broad-leaved trees." **Kirsten and Topp (1991)**, 24
"The synchrony between the emergence of larval winter moth (*Operophtera brumata* L.) and budburst of pedunculate oak (*Quercus robur* L.), its primary host (Wint, 1983), is critical for the moth's success." **Buse and Good (1996)**, 171
"*Operophtera brumata* L. [is] one of the most important herbivores of oak." **Dongen *et al.* (1997)**, 139
"*Operophtera brumata* L. (Lepidoptera: Geometridae) is one of the most abundant insect herbivores on *Q. robur*." **Tikkanen and Julkunen-Tiitto (2003)**, 174
"*Operophtera brumata* is a polyphagous moth that is able to feed on a range of tree and shrub species (Holliday, 1985). In the U.K., oak *Quercus* spp. (Fagaceae) and other deciduous trees are regarded as the optimal hosts of this insect (Feeny, 1970; Wint, 1983; Holliday, 1985), but *O. brumata* has also been recorded on heather *Calluna vulgaris* (Ericaceae) where it reaches outbreak densities (Picozzi, 1981; Kerslake *et al*., 1996)." **Vanbergen *et al.* (2003)**, 55
"Timing of egg hatching in *O. brumata* is itself under selection for synchronization with bud burst in oak trees (*Quercus robur*). Caterpillars of this moth rely on oak leaves for food, and although the oak trees have been opening their buds earlier, advancement in the date of egg hatching has been more extreme."

“Our fitness estimations are based on the assumption that the optimal moment of egg hatch is determined by the moment of *Q. robur* bud burst. *O. brumata* is not a specialist species: it can also feed on leaves from tree species other than oak.”
van Asch *et al.* (2007), 252

“Pedunculate oak, *Quercus robur*, is a favoured host of winter moth”
Singer and Parmesan (2010), 324

“However, caterpillars [of the winter moth] are available to the birds only during a brief period in spring, as the larvae exploit the newly emerged leaves of their host trees (predominantly oak *Quercus* spp.; Varley and Gradwell 1958)”
Hinks *et al.* (2015), 68

“Pedunculate oak is described as the primary host plant of winter moth throughout its native range.”
O’Donnell *et al.* (2019), n/a

Table S2. Taxonomic coverage of the literature on winter moth caterpillar performance across host-plants. Studies are grouped by those which consider the effects of varying degrees of asynchrony on performance across host-plant species and those which consider performance at one time point only. Particularly in studies of synchrony, there is a clear bias towards oak *Quercus*. The inclusion of a host-plant species in a particular study is indicated by ✓. Taxa arranged alphabetically within higher groupings. Performance Metrics used are: S (survival); P (pupal mass); GR (growth rate); DI (development index); DT (development time); FAb (abundance across host-plants measured in the field); LP (larval choice experiment); FU (food utilisation or assimilation rate); FP (frass production); F (fecundity); and MO (mass of ova).

				ADOXACEAE	**ERICACEAE**				**BETULACEAE**											**FAGACEAE**									**GROSSULARIACEAE**	**SALICACEAE**												**MALVACEAE**		**OLEACEAE**	**PINACEAE**	**RHAMNACEAE**	**ROSACEAE**										**ULMACEAE**	**SAPINDACEAE**		
				Sambucus	Calluna	Vaccinium			Alnus		Betula						Carpinus	Corylus	Ostrya	Fagus	Quercus								Ribes	Populus	Salix											Tilia		Fraxinus	Picea	Rhamnus	Crataegus	Malus				Prunus			Rubus	Sorbus	Ulmus	Acer		
First Author	**Year**	**Country**	**Performance Metric**	racemosa	vulgaris	angustifolium	corymbosum	myrtillus	incana	glutinosa	spp	nana	papyrifera	pendula	pubescens	pubescens ssp. czerepanovii	betulus	avellana	virginiana	sylvatica	afares	canariensis	cerris	garryana	pubescens	robur	rubra	suber	spp.	tremula	alba alba	alba vitellina	aucuparia	caprea	fragilis	myrsinifolia	pentandra	phylicifolia	triandra	viminalis	x rubens	americana	cordata	excelsior	sitchensis	frangula	monogyna	domestica	sylvestris	sp. (crab apple)	spp.	padus	pensylvanica	spinosa	idaeus	aucuparia	americana	platanoides	pseudoplatanus	rubrum
Performance effects of asycnhrony examined																																																												
Feeny	1970	UK	P																							✓																																		
Wint	1983	UK	S, P, DT, LP															✓		✓						✓																					✓		✓					✓						
Watt	1991	UK	S																																										✓															
Hunter	1991	UK	P																																										✓															
Kirsten	1991	Germany	P, GR, F																							✓					✓	✓		✓	✓				✓	✓	✓																			
Kerslake	1997	UK	S, P, GR, DI		✓																																																							
Buse	1999	UK	P																							✓																																		
Tikkanen	2002	Finland; Denmark	P, DT																							✓																										✓								
Tikkanen	2003	UK	S																							✓																																		
Van Dongen	2006	Belgium	P																							✓																																		
van Asch	2007	Netherlands	S, P																							✓																																		
Wesołowski	2008	Poland	FAb																							✓																																		
Kulfan	2018	Slovakia	FAb																			✓		✓																																		
Performance at only one time point considered																																																												
Cuming	1961	Canada	FAb																✓								✓																✓									✓						✓			✓
Warrington	1985	UK	P, GR																																																								✓	
Roland	1987	Canada	P																				✓																									✓												
Kerslake	1996	UK	S, P GR, DI		✓			✓																																																				
Kerslake	1998	UK	S, P GR, DI		✓																																																							
Buse	1998	UK	P, DT, FU, FP, F, MO																							✓																																
Lavola	1998	Russia	LP											✓																																						✓								
Tikkanen	1998	Russia	FAb	✓					✓						✓											✓			✓	✓			✓	✓												✓						✓						✓		
Tikkanen	1999	Russia	S, FAb	✓					✓					✓	✓			✓								✓			✓	✓			✓	✓		✓		✓								✓						✓			✓	✓		✓		
Tikkanen	2000	Finland	S, P, GR, DT																							✓				✓								✓														✓								
Ruuhola	2001	Finland	GR																																	✓	✓	✓																						
Vanbergen	2003	UK	S		✓																					✓																			✓															
Strengbom	2005	Sweden	S, P					✓																																																				
Wesołowski	2006	Poland	FAb							✓	✓						✓	✓								✓				✓													✓	✓														✓		
Belsing	2015	Norway	S, P					✓				✓				✓																																												
Pepi	2016	Canada	S																								✓																					✓												✓
Mannai	2017	Tunisia	S, DT, FAb																		✓	✓						✓																																
O'Donnell	2019	USA	S, P, GR, FAb			✓	✓						✓														✓																					✓		✓			✓							✓

Table S3. Establishment dates of caterpillar cultures on each host-plant species assayed in the experiment. The phenology of each host-plant species differed in the field. For each experiment "Time 0" was taken as the timing of the first small leaves breaking/unfurling, with a recognisable shape. The treatment groups for each host-plant species therefore began on different calendar dates, as indicated above. Variation in the calendar date timing of each treatment was minimised as far as possible, so as not to confound host-plant effects with any effect of hatch timing, but was limited based on the observed phenology of each species in the field. The latest treatments were begun several days after the earliest treatments.

Host-plant Species	**Date of Establishment**
Acer	1 May
Alnus	29 Apr
Betula	1 May
Crataegus	25 Apr
Malus	29 Apr
Prunus	29 Apr
S. alba	29 Apr
S. caprea	2 May
Quercus	4 May

Table S4. Modelling the effects of host-plant on winter moth caterpillar performance, measured as survival to pupation, pupal mass, and development time. Justification gives the hypothesis tested by each term. A significant Host-plant:Population interaction would be consistent with local adaptation (though not proof of it)—a significant effect of population, on the other hand, would perhaps indicate flaws in the experimental procedure, particularly if the performance was highest in the population most proximate to the rearing site (Edinburgh). ^ indicates as above.

Response Variable	**Random Effect Term**	**Justification**
Surv/Dev. Time	Host-plant	Tests whether performance is consistently higher on certain host-plant species across all four populations
	Population	Tests whether performance is consistently higher in caterpillars from certain populations across all host-plant species. This shows whether, for example, individuals from some populations are performing consistently better in the common garden environment (e.g. perhaps populations closer to that site would perform better than those collected from farther away, due to, e.g., adaptation to weather conditions, clines in host-plant traits, etc.)
	Host-plant:Population	Tests whether performance on certain host-plants is population specific, i.e. do caterpillars from one population perform better on a particular host-plant species that those from another population
	Rearing Culture	Tests whether performance is consistently higher in individuals reared in the same captive environment, a “culture”
Mass	Host-plant	^
	Population	^
	Host-plant:Population	^
	Sex	Tests whether performance is consistently higher in one sex compared with the other
	Rearing Culture	^

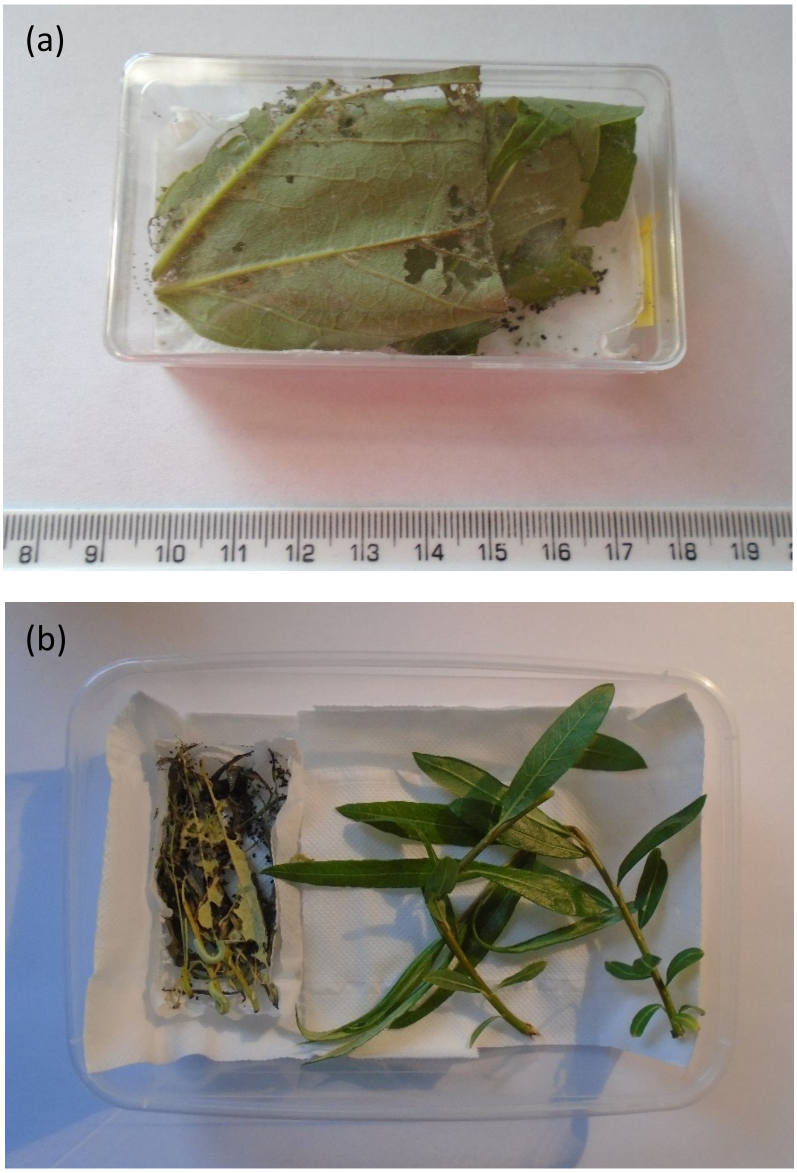

Figure S1. Caterpillar rearing containers. Caterpillars were housed when neonates in (a) small 75 x 50 x 15mm transparent plastic containers. When large enough, usually in the third instar, caterpillars were transferred to (b) larger 175 x 100 x 50mm disposable plastic food containers. Rearing containers were lined with tissue which was replaced each time new food was added. When new food was added at one end of the container the old food was left in to allow caterpillars to move onto the fresh plant material as and when they chose. Similarly, (b) when caterpillars were transferred to larger rearing containers, the whole contents of the smaller container were moved, and new food supplied for caterpillars to move off onto as they chose. Scale bar in (a) is in centimetres and millimetres.

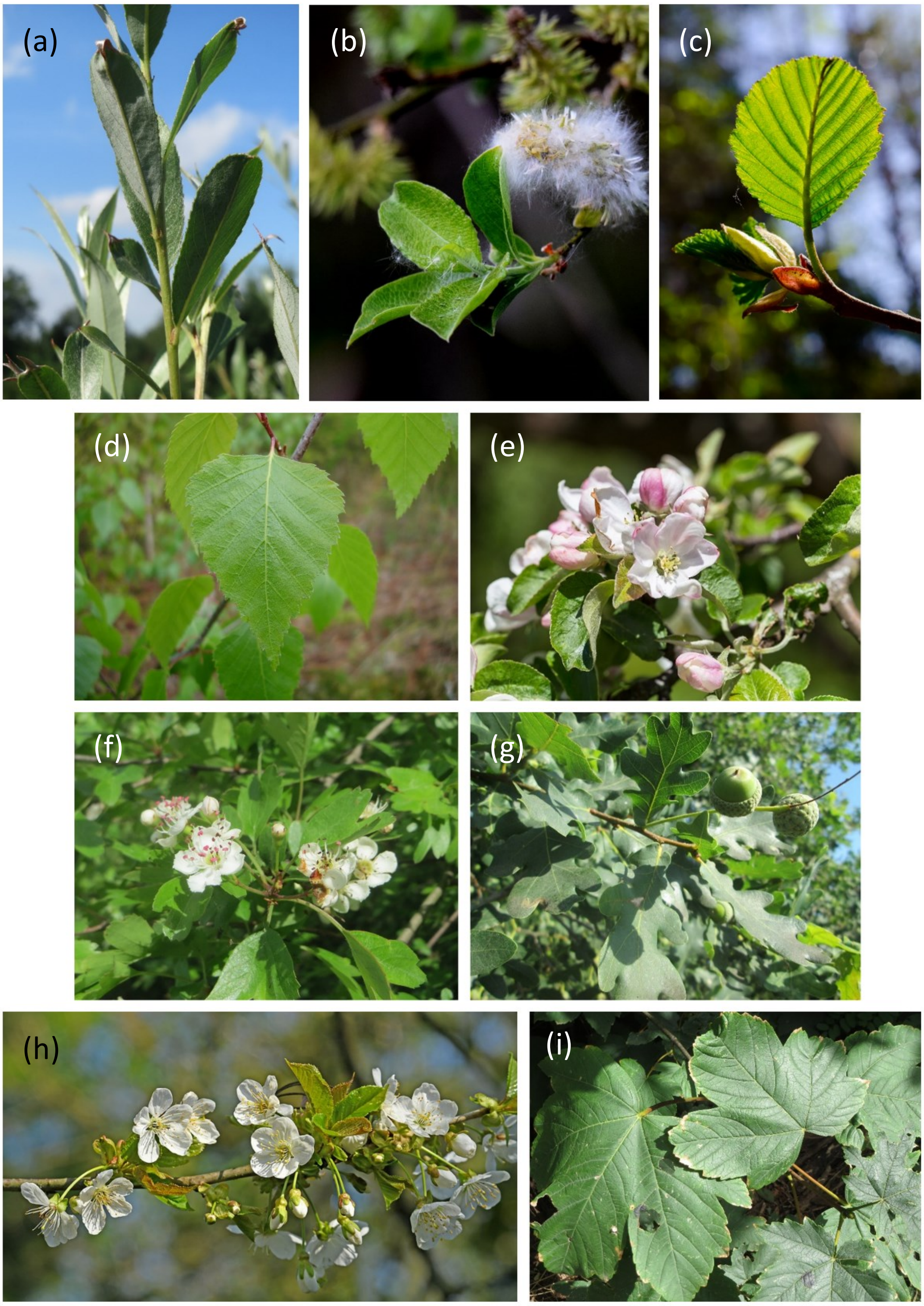

Figure S2. Host-plant species studied in the experiment. (a) Willow *Salix alba*, (b) Sallow *Salix caprea*, (c) Alder *Alnus glutinosa*, (d) Birch *Betula pendula*, (e) Apple Malus domestica, (f) Hawthorn *Crataegus monogyna*, (g) Oak *Quercus robur*, (h) Cherry *Prunus avium*, (i) Sycamore *Acer pseudoplatanus*. Image credits: Andreas Rockstein (a, f, g, I), Giuseppe Morlando (b), Tero Laakso (c), dragonfly201011 (d), Mariya Novikova (e), Karin Rogmann (h). Taken from Flickr.org, reproduced under a creative commons licence.

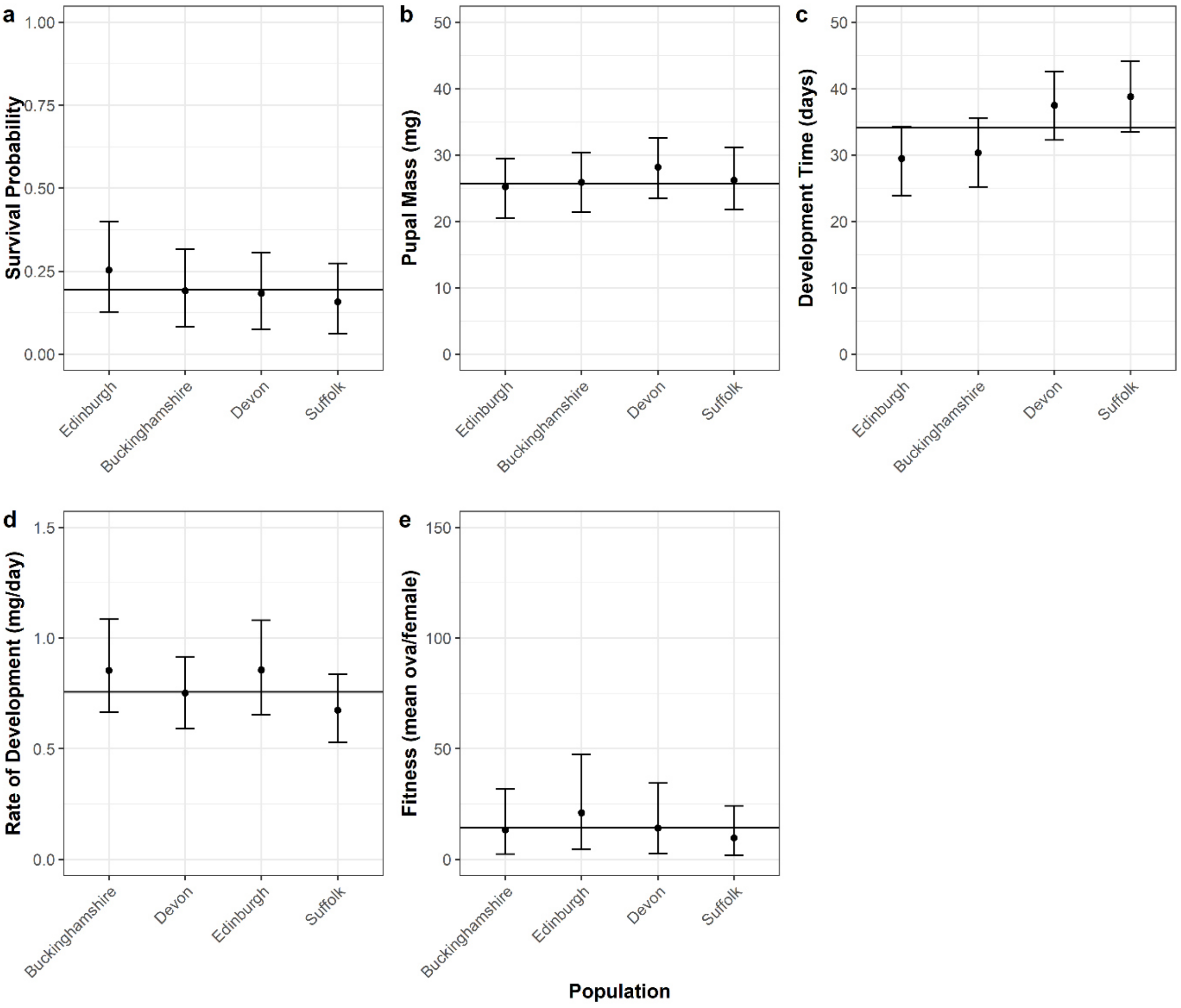

Figure S3. Overall winter moth performance in different populations across host-plants. Performance quantified as (a) survival probability, (b) pupal mass, (c) development time, (d) rate of development, and (e) estimated fitness. Mean estimates and 95% credible intervals shown. Global mean for each performance metric shown by solid line.

APPENDIX 1

Appendix 1: Pupal mass, fecundity, and estimating fitness in the winter moth *Operophtera brumata* (Lep.: Geometridae)

Introduction

Pupal mass in insects can be a reliable predictor of adult female fecundity (Heisswolf et al., 2009) and is therefore a very tractable measure of performance under different conditions—larger larvae produce larger pupae which yield larger females containing greater quantities of ova. Indeed, in the sizeable literature on the ecology and biology of the winter moth, variation in pupal mass across treatment groups is frequently interpreted as indicative of differences in fitness (see for example: Feeny, 1970; Wint, 1983; Kirsten and Topp, 1991; Tikkanen and Lyytikäinen-Saarenmaa, 2002; Belsing, 2015). In addition, if pupal mass falls below a certain level viability may be affected (Wint, 1983). Across insect species, both the slope of the mass-egg relationship (the correlation coefficient) and the intercept can vary—and can be affected within a species by environmental conditions.

In analysing this relationship, it is possible that we might find these parameters differing, plastically or adaptively, within a species, under different conditions, to optimise fitness (for example, by producing more ova per unit mass on a given host-plant). Methodologically there is further a distinction to be drawn between potential fecundity (the number of mature oocytes or eggs in the reproductive tract) and realised fecundity (the number of fertile ova laid by a female during her lifetime)—the relationship between pupal mass and the latter is often far less precise (see for example Heisswolf *et al*., 2009). In the winter moth, only three studies have attempted to quantify the mass-fecundity relationship experimentally. Both Holliday (1977) and Rubtsov and Utkina (2011) trapped wild female moths in winter on tree trunks and measured their fresh mass and potential fecundity. The data obtained by the former are also reported by Singer and Parmesan (2010), though mistakenly attributed to a later paper (Holliday, 1985). Roland and Myers (1987) also trapped and weighed wild caught females, then back-calculated pupal mass, and estimated potential fecundity by dissection.

Here, I analysed the relationship between fecundity and pupal mass in the winter moth using my own data and that reported by the other studies described above. I tested if the relationship varied by population and if it was affected by the host-plant on which the caterpillar was reared. From this relationship I show how a metric of fitness can be estimated when caterpillar survival to pupation and pupal mass are both measured.

Pupal mass as a predictor of fecundity, and the effects of geographical and trophic factors

Methods

I measured the fresh mass of winter moth pupae raised in this captive rearing experiment one month after pupation (+/- 5 days). Pupae were then stored at 21°C until 1 September 2020, when they were placed outdoors in ambient temperature (Stirlingshire, UK; 56.069°N, -3.767°E) in sealed containers under an open canopy which provided shade. After female eclosion later in winter (December), they were killed in 75% ethanol and dissected. The potential fecundity of each individual was measured (no. oocytes/female). To analyse the mass-fecundity relationship in these data I fitted a general linear mixed effects model in the *R* v. 4.0.3 package MCMCglmm (Hadfield, 2010). I tested for differences in the mass-fecundity relationship among host-plants by allowing for a random slope and intercept across host-plants (Model 1. Response: Fecundity; Explanatory Fixed effects: Pupal Mass, Population; Random effect: Pupal Mass:Host-plant, with random slopes. Default priors, 500000 iterations with 250000 burn-in, thinning every 50 iterations). Pupal mass was mean centred at 25mg.

In addition to my own data, I used the *R* package metaDigitise (Pick et al., 2018) to extract the mass-fecundity data from the relevant figures in three previous papers which measured the fecundity and mass of winter moth individuals (Holliday, 1977; Roland and Myers, 1987; Rubtsov and Utkina, 2011). I combined these data with my own to generate an overall model and test for: (i) any differences in the mass-fecundity relationship between winter moth populations at different geographical sites; and, (ii) any significant difference in the mass-fecundity relationship between studies using adult female mass or pupal mass as proxies for fecundity (Model 2. Response: Fecundity; Explanatory Fixed effects: Pupal Mass, Dataset; Random effects: Host-plant. Default priors, 500000 iterations with 250000 burn-in, thinning every 50 iterations).

Results and discussion

Those studies which measure female mass, rather than pupal mass, unsurprisingly find that predicted fecundity at a mass of 25mg (the approx. mean pupal mass) is significantly higher (Holliday = 174.41 ova/female, CIs: 163.92, 184.67; Rubtsov and Utkina = 159.43, CIs: 154.18, 164.26 vs Roland and Myers = 140.23, CIs: 133.53, 164.26; Weir = 143.68, CIs: 139.24, 148.12) (Figure A1.1). This may be accounted for by individuals undergoing a loss of mass either during the period of development from summer to eclosion in winter (e.g. through water loss) or during the process of eclosion itself, which involves shedding the pupal case. As pupal/female mass increases in my data, fecundity increases significantly (slope = 9.81, CIs: 9.26, 10.34). The mass-fecundity slope does not differ significantly between my study and the other datasets (Holliday = 10.20, CIs: 9.46, 10.99; Roland and Myers = 9.57, CIs: 8.88, 10.25; Rubtsov and Utkina = 9.46, CIs: 9.04, 9.89).

Taking my own data separately, point estimates of the among host-plant species variance in the intercept (26.24, CIs: 0.00, 108.51; intercept mean-centred at 25mg) and slope (0.86, CIs: 0.00, 3.16) of the mass-fecundity relationship are small and the lower bounds of the credible intervals approach zero, suggesting these are non- or marginally significant. Pupal or adult female mass is therefore a consistent predictor of potential fecundity, with no significant geographical or host-specific variation across these data (Figure A1.1).

Estimating fitness in the winter moth

Factors such as survival, pupal mass, or development time are informative with regard to caterpillar performance on different host-plants, but are only a few of the many facets affecting overall fitness. Interpreting how these interact can be difficult: how does performance on a host species which yields high mortality but high pupal mass compare with one resulting in low mortality but low final pupal mass? Ideally, we could measure overall fitness in the field in each case, or as close an approximation to this as possible, to infer which is the optimal host-plant. Below, I develop a metric of absolute fitness in the winter.

Assuming the pupal mass-fecundity relationship is linear, we can predict the fecundity of a moth—a more reliable signpost of fitness—from its pupal mass using the standard equation of a straight line:

$$y = mx + c$$

As:

$$\text{Fecundity} = m_{\text{mf}} * \text{Mass} + c_{\text{mf}}$$

Where m_{mf} is the posterior of the slope of pupal mass and potential fecundity found in my own data, Mass is the pupal mass in a given treatment group, and c_{mf} is the intercept of the mass-fecundity relationship. Using the posterior distributions of each of these terms from Bayesian models of my own data, I obtain a posterior for the predicted fecundity of an individual for a given pupal mass.

I then combine the predicted fecundity and the posterior of the probability of survival to give an estimate of fitness:

$$\text{Fitness} = \text{Fecundity} * \text{Probability of Survival}$$

Or, more completely:

$$\text{Fitness} = (m_{\text{mf}} * \text{Mass} + c_{\text{mf}}) * (\text{Survival})$$

The estimate of absolute fitness here is therefore given as the *predicted ova per female in a given treatment group*. From the posterior distributions it is possible to calculate a mean value and 95% HPD interval on this estimate of fitness.

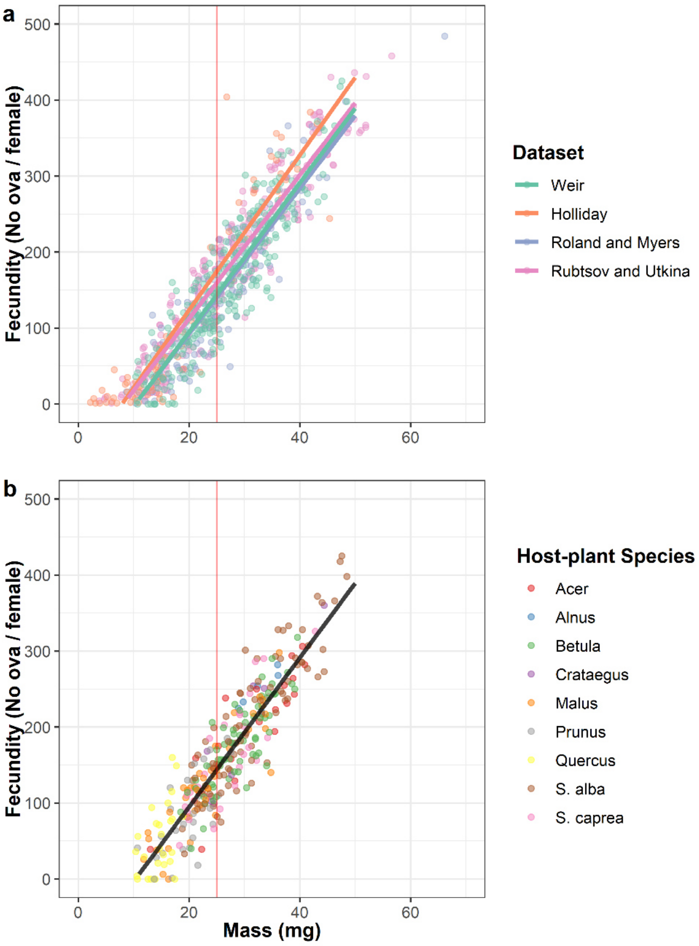

Figure A1.1. Relationship of winter moth pupal mass to potential female fecundity across (a) different studies and (b) across different host-plants in my own data. Regression line shows mean estimates for (a) each study (Slopes: Weir = 9.81, CIs: 9.26, 10.34; Holliday = 10.20, CIs: 9.46, 10.99; Roland and Myers = 9.57, CIs: 8.88, 10.25; Rubtsov and Utkina = 9.46, CIs: 9.04, 9.89) and (b) overall in the data from this study (slope = 9.81, CIs: 9.23, 10.32). Data in (a) were derived from Weir (this study), Holliday (1977), Roland and Myers (1987), and Rubtsov and Utkina (2011). Weir and Roland and Myers report fresh pupal mass, while Holliday and Rubtsov and Utinka use fresh female mass. Data in (b) were derived solely from the present study.

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APPENDIX 2

Appendix 2: The effects of mixed brood cultures on the analysis of caterpillar performance and geographical divergence

A weakness of the experimental design employed in this study was that, for the sake of logistical simplicity, several offspring from a single brood (all offspring of one female) were reared across each mixed brood culture of 20 individuals. This made it impossible to later identify which individual belonged to which brood. Caterpillars from the same brood will likely be more similar to one another in terms of their performance across host-plants than to other members of the population, due to genetic similarity and maternal effects.

In the context of the experimental design and analysis presented here, the variance for a given population may be under-estimated, relative to the real value in nature, because some of the individuals within each culture are related. And, since individual identity is not tracked, this is cannot be controlled for in the statistical analysis. In addition, where similar brood compositions are used across different experimental treatments it may be expected to bias effects towards the null (i.e. no effect). Alternatively, biological processes such as diversified bet-hedging among the offspring of individual females may mean that among brood variances are small relative to among offspring differences—in this case, we would not expect the results to be biased in the way outlined above.

For analyses conducted at the culture level (e.g. survival) it is possible to include the contributing broods as a multi-membership random term and to estimate the brood effect, but for analyses that must be conducted at the individual level (e.g. mass at pupation) this cannot be done. Therefore, to assess the sensitivity of the statistical inferences presented in this study to brood effects of different magnitudes I conducted extensive simulations.

To recap, the two main aims of this study were:

a) To quantify variation in caterpillar performance among nine host-plant species
b) To quantify host-by-population variation in caterpillar performance (suggesting geographical divergence)

Based on the anticipated impact of among-brood variance on model parameters, it follows that if the variation in performance among broods is substantial then this design and analysis will tend to have a high false positive rate for detecting among population differences, and reduced power to detect among host-plant and host-by-population effects. The aim of the simulations presented here was to assess the sensitivity of these effects, under the focal experimental design, with different magnitudes of among-individual and among-brood variance.

Simulated data and analyses

To explore the implications of the experimental design presented here on the expected outcome of the analyses (variance estimates and false positive rates), I generated a simulation of the study in *R* (assuming a Gaussian response) and manipulated the variance of each of the main parameters. The data and model structure followed that used in the study, including the difference in brood number between Edinburgh and the remaining three populations. Across simulations I assessed the sensitivity of inferences to different magnitudes of variance among individuals, broods, populations, host-plants, and host-by-population interactions (Table A2.1). The among culture variance was set at 0.005 for all simulations. The simulation code is provided at the end of this appendix. For each parameter combination I conducted 500 simulations. For each of the variance terms that are critical to the hypotheses being tested (population, host-plant, host-by-population) I quantified: the median variance estimate across simulations to assess bias; and, the proportion of simulations returning a significant p-value (< 0.05, based on a likelihood ratio test) to assess the false positive (type I error) rate.

From these simulations we can see that under certain circumstances the design can produce a high rate of false positive effects of population (Fig. A2.1). However, this only occurs in a limited set of situations, with the severity of the issue increasing as the among-brood variation increases relative to among-individual variation. Variance estimates have broad confidence intervals, but are generally not significantly different from the true value in each simulation. For the remaining parameters—including, crucially, the host-plant by population interaction term—the design and model are very conservative. This is even the case when among-brood variation is much greater than among-individual variation (a situation which I think unlikely to be the case in nature, and which is inherently more likely, under this design, to produce spurious population effects).

Conclusions

Although these simulations suggest that, under some circumstances, the experimental design is prone to false positives for an effect of population, none of the results presented in this paper show such an effect. More importantly, the simulations demonstrate that the design and model generate very conservative estimates of the variance and significance of both the effect of host-plant species (**Aim A**) and the host-plant by population interaction (**Aim B**). This evidence, taken collectively, demonstrates that this experimental design is appropriate for addressing the stated aims of this study.

Table A2.1. Parameters used in simulations of the study design. Each combination ('Scenario') of among population, among host-plant, and host-plant by population variance used in the simulations are assigned a lettered code, referred to in Figure A2.1.

Variance						
Among individual	Among females/ broods	Among rearing cultures	Among populations	Among host-plants	Host-plant: population inter.	Scenario
1	1	0.05	0	0	0	*a*
1	1	0.05	10	0	0	*b*
1	1	0.05	0	10	0	*c*
1	1	0.05	0	0	10	*d*
1	1	0.05	10	10	0	*e*
1	1	0.05	0	10	10	*f*
1	1	0.05	10	0	10	*g*
1	1	0.05	10	10	10	*h*
10	1	0.05	0	0	0	*a*
10	1	0.05	10	0	0	*b*
10	1	0.05	0	10	0	*c*
10	1	0.05	0	0	10	*d*
10	1	0.05	10	10	0	*e*
10	1	0.05	0	10	10	*f*
10	1	0.05	10	0	10	*g*
10	1	0.05	10	10	10	*h*
1	10	0.05	0	0	0	*a*
1	10	0.05	10	0	0	*b*
1	10	0.05	0	10	0	*c*
1	10	0.05	0	0	10	*d*
1	10	0.05	10	10	0	*e*
1	10	0.05	0	10	10	*f*
1	10	0.05	10	0	10	*g*
1	10	0.05	10	10	10	*h*
10	10	0.05	0	0	0	*a*
10	10	0.05	10	0	0	*b*
10	10	0.05	0	10	0	*c*
10	10	0.05	0	0	10	*d*
10	10	0.05	10	10	0	*e*
10	10	0.05	0	10	10	*f*
10	10	0.05	10	0	10	*g*
10	10	0.05	10	10	10	*h*

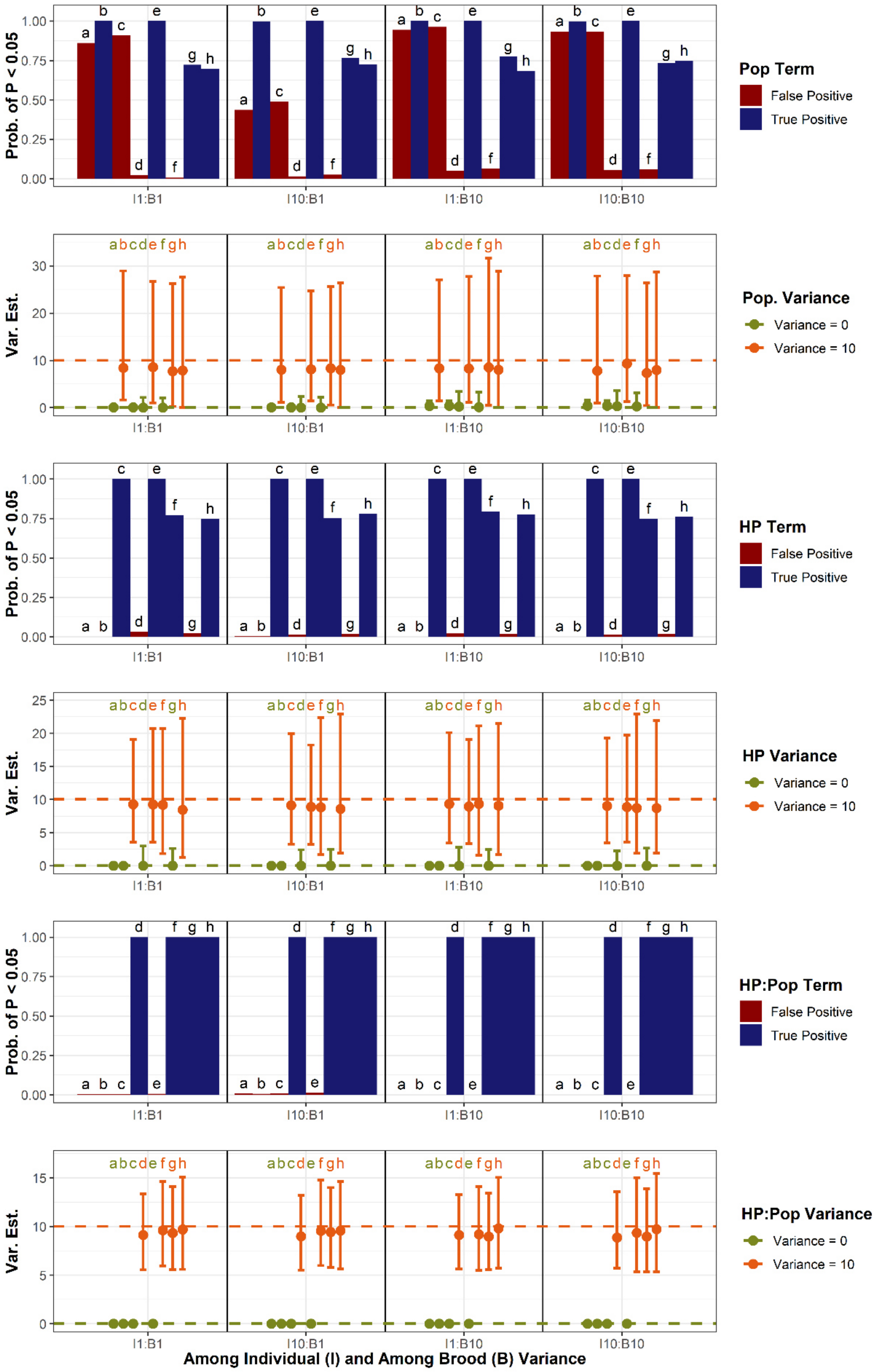

Figure A2.1. True and false positive rate of term significance and the variance estimates (±95%) in simulated analyses, shown for Population term (Pop), Host-plant term (HP), and Host-plant by population interaction term (HP:Pop). For details of each simulated scenario (lettered *a* to *h*), see Table A2.1. Each scenario is shown simulated with four levels of among-individual and among-brood variance.

Annotated R Code for Simulations

```
##Step 1 – Set-up the structure of the experiment and dataset
population<-as.factor(rep(rep(1:4,each=100),9))
#population1==Edi
tray<-as.factor(rep(1:180,each=20))
hostplant<-as.factor(rep(1:9,each=400))
female<-rep(NA,3600)
female[1:100]<-female[401:500]<-female[801:900]<-female[1201:1300]<-female[1601:1700]<-
female[2001:2100]<-female[2401:2500]<-female[2801:2900]<-female[3201:3300]<-1:100

#Set-up female sampling design for the other trays (i.e. ‘cultures’) and sites. The same female
structure across all trays and host-plants

females2<-c(126:141,126:129)
females3<-c(142:155,142:147)
females4<-c(156:174,156)
plusx<-100
female[plusx+1:100]<-female[plusx+401:500]<-female[plusx+801:900]<-female[plusx+1201:1300]<-
female[plusx+1601:1700]<-female[plusx+2001:2100]<-female[plusx+2401:2500]<-
female[plusx+2801:2900]<-female[plusx+3201:3300]<-rep(females2,5)
plusx<-200
female[plusx+1:100]<-female[plusx+401:500]<-female[plusx+801:900]<-female[plusx+1201:1300]<-
female[plusx+1601:1700]<-female[plusx+2001:2100]<-female[plusx+2401:2500]<-
female[plusx+2801:2900]<-female[plusx+3201:3300]<-rep(females3,5)
plusx<-300
female[plusx+1:100]<-female[plusx+401:500]<-female[plusx+801:900]<-female[plusx+1201:1300]<-
female[plusx+1601:1700]<-female[plusx+2001:2100]<-female[plusx+2401:2500]<-
female[plusx+2801:2900]<-female[plusx+3201:3300]<-rep(females4,5)
female<-as.factor(female)
hostbypop<-as.factor(as.numeric(as.factor(paste(hostplant, population))))

##Step 2 – Set-up parameters.

#generate model data-frame to fill in with results of sims
sims<-data.frame(ind=numeric(), brood=numeric(), culture=numeric(), pop=numeric(),
hp=numeric(), hp.pop=numeric(), fp_pop=numeric(), fp_hp=numeric(), fp_hp.pop=numeric(),
var_m_pop=numeric(),  var_m_hp=numeric(), var_m_hp.pop=numeric(), var_l_pop=numeric(),
var_u_pop=numeric(), var_l_hp=numeric(), var_u_hp=numeric(), var_l_hp.pop=numeric(),
var_u_hp.pop=numeric())

#load data-frame of test variances to plug into models, see attached file sim_data#
#set-up row no. from data-frame of test variances
r<-1

#specify parameters used for each component of simulation, drawing values from each row in
sim_data
individualvar<-sim_data[r,1]
femalevar<-sim_data[r,2]
trayvar<-sim_data[r,3]
populationvar<-sim_data[r,4]
```

```
hostplantvar<-sim_data[r,5]
hostbypopulationvar<-sim_data[r,6]

##Step 3 - Simulate data

resp<-
0+rnorm(4,0,sqrt(populationvar))[population]+rnorm(9,0,sqrt(hostplantvar))[hostplant]+rnorm(36,0,
sqrt(hostbypopulationvar))[hostbypop]+
rnorm(174,0,sqrt(femalevar))[female]+rnorm(180,0,sqrt(trayvar))[tray]+rnorm(3600,0,sqrt(individualvar))

##Step 4 - Run model
library(lme4)
#model<-lmer(resp~1+(1|population)+(1|hostplant)+(1| hostbypop)+(1|tray))
#summary(model)

#run 500 simulations
#create places to store accumulated data from repeated runs of model

pval_population<-c()
pval_hostplant<-c()
pval_hostbypop<-c()
varpop<-c()
varhost<-c()
varhostbypop<-c()

for (sim in 1:500){

resp<-
0+rnorm(4,0,sqrt(populationvar))[population]+rnorm(9,0,sqrt(hostplantvar))[hostplant]+rnorm(36,0,
sqrt(hostbypopulationvar))[hostbypop]+
rnorm(174,0,sqrt(femalevar))[female]+rnorm(180,0,sqrt(trayvar))[tray]+rnorm(3600,0,sqrt(individualvar))

model<-lmer(resp~1+(1|population)+(1|hostplant)+(1| hostbypop)+(1|tray))
model_nointer<-lmer(resp~1+(1|population)+(1|hostplant)+(1|tray))
model_nohost<-lmer(resp~1+(1|population)+(1| hostbypop)+(1|tray))
model_nopop<-lmer(resp~1+(1|hostplant)+(1| hostbypop)+(1|tray))

pval_hostbypop[sim]<-anova(model, model_nointer)["Pr(>Chisq)"][[1]][2]
pval_hostplant[sim]<-anova(model, model_nohost)["Pr(>Chisq)"][[1]][2]
pval_population[sim]<-anova(model, model_nopop)["Pr(>Chisq)"][[1]][2]

varhostbypop[sim]<-VarCorr(model)$hostbypop[1]
varhost[sim]<-VarCorr(model)$hostplant[1]
varpop[sim]<-VarCorr(model)$population[1]
}

par(mfrow=c(3,2))
```

```
##Plotting each effect, evaluating median estimates relative to true value, and false/true positive
rates

hist(pval_population)
hist(varpop)
abline(v= populationvar,col="red")

hist(pval_hostplant)
hist(varhost)
abline(v= hostplantvar,col="red")

hist(pval_hostbypop)
hist(varhostbypop)
abline(v= hostbypopulationvar,col="red")

##To calculate proportion f/positives
sims[nrow(sims) + 1,] = c(individualvar, femalevar, trayvar, populationvar, hostplantvar,
hostbypopulationvar, sum(pval_population<0.05)/500, sum(pval_hostplant<0.05)/500,
sum(pval_hostbypop<0.05)/500, median(varpop), median(varhost),
median(varhostbypop), quantile(varpop, c(0.05, 0.95)), quantile(varhost, c(0.05, 0.95)),
quantile(varhostbypop, c(0.05, 0.95)))
```