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## Adaptation during biological invasions and the case of *Adelges tsugae*

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### ABSTRACT

**Question:** Do invasive species adapt during range expansion? Data are few and many expect adaptation to be constrained by low genetic variation in invaders, which frequently experience population bottlenecks during colonization.

**Experimental results:** We used a common-garden experiment to show that the invasive hemlock woolly adelgid (Insecta: Hemiptera: Adelgidae: *Adelges tsugae*) has evolved greater resistance to cold shock as it has expanded its range northward. This insect feeds on exposed twigs in winter and is vulnerable to extreme cold.

**Modelling results:** *Adelges tsugae* has grown to a sufficiently large population size that its adaptive evolution appears unconstrained by the availability of new mutants, despite its parthenogenetic reproduction. Conservatively, its population size likely exceeds  $1/(2u)$  within 40 ha of forest, where  $u$  is the haploid per-base mutation rate, so that on average a mutation arises each generation at every base pair in the entire genome within this area.

**Conclusion:** This escape from genetic constraint is likely to be found in many species that have invaded successfully, facilitating their adaptation to novel conditions.

*Keywords:* adaptation, asexual reproduction, cold tolerance, eastern hemlock, environmental extremes, hemlock woolly adelgid, invasive species, mutation limitation, *Tsuga canadensis*.

### INTRODUCTION

Invasive species can cause severe ecological and economic impacts, so it is of primary importance to understand the attributes that permit them to spread (Baker and Stebbins, 1965; Sakai *et al.*, 2001). Considerable attention has been devoted to identifying ecological correlates of range expansion (reviewed by Sakai *et al.*, 2001), but much less is known about adaptive evolutionary changes that might be occurring as invasion proceeds (Sakai *et al.*, 2001; Lee, 2002). From first principles, it is unlikely that introduced species will be at the optimal phenotype in their new fitness landscapes, so adaptation during colonization and range expansion is

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expected (Blossey and Nötzold, 1995; Müller-Schärer *et al.*, 2004). The extent and rate of such evolutionary adjustments will depend not only on the degree of discrepancy between the invader's native and exotic environments (García-Ramos and Rodríguez, 2002), but also on the genetic diversity and architecture of its founding population (Ellstrand and Schierenbeck, 2000), and many of the best examples of rapid evolutionary change involve invasive species (Reznick and Ghalambor, 2001). Traits associated with dispersal and colonization ability are predicted to be subject to evolution during range expansion (Travis and Dytham, 2003), and patterns consistent with this prediction are observed in the invasive tree, *Sapium sebiferum* (Seimann and Rogers, 2001).

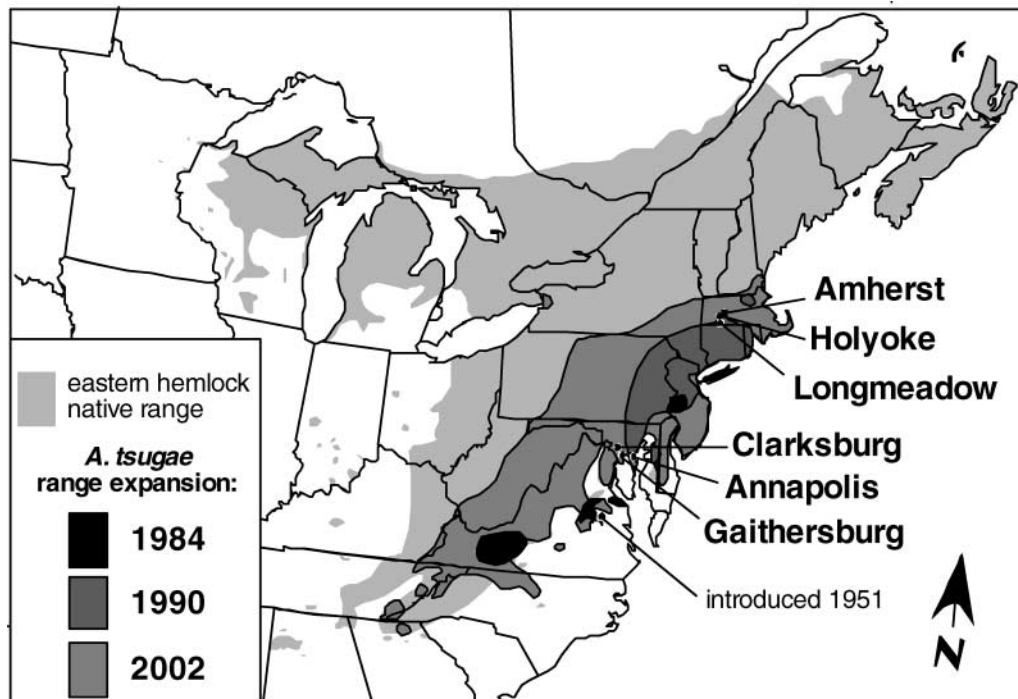
One class of traits in invasive species that appears especially prone to evolve is their tolerance of abiotic environmental extremes. While success in the primary stages of invasion depends substantially on how well the abiotic average conditions match in the native and introduced ranges (Baker and Stebbins, 1965), environmental extremes vary on different spatio-temporal scales (Easterling *et al.*, 2000; Meehl *et al.*, 2000), are less predictable (Palmer and Ralsanen, 2002), and are therefore less likely to match perfectly. Extreme conditions can generate high, density-independent mortality in the invading population, providing strong selection coefficients on genetic variation for tolerance (Lytle, 2001), and physiological systems providing stress tolerance readily adapt to local conditions (Feder and Hofmann, 1999). Ultimately, abiotic extremes will be so harsh as to preclude survival, and this will delimit the geographic extent of the invasion, just as abiotic conditions often determine the limits to the ranges of native species (e.g. Jenkins and Hoffmann, 1999; Magiafoglou and Hoffmann, 2003). Environmental extremes also vary temporally and geographically within native ranges. We can therefore expect genetic variation for tolerance to extremes to exist in the native populations from which founders are drawn, especially where surviving extreme conditions engenders trade-offs with other fitness components. For example, survival of extreme drought conditions in Darwin's finches is favoured by beak shape and body size characteristics that are mildly disadvantageous under normal rainfall conditions; temporal variation in environmental extremes helps maintain quantitative genetic variance in the population (Grant and Grant, 2002).

Invasive species often have traits that are conducive to rapid evolution. Life-history traits that predispose exotic species to be effective invaders include small body size, high fecundity, short maturation time and phenotypic plasticity (Kolar and Lodge, 2001; Sakai *et al.*, 2001). All are also correlates of rapid population growth and the maintenance of large population densities during favourable periods. These attributes allow genetic variance to build rapidly by drift and spatiotemporally varying selection during the population expansion process. Standing genetic variance and the ability to recover to large population sizes following perturbations are in turn prerequisites for rapid adaptive evolutionary change under strong natural selection (Gould, 1991; Reznick and Ghalambor, 2001).

As a test case for the prediction that environmental extremes should drive significant adaptations in invasive species, we consider the hemlock woolly adelgid, a forest pest in eastern North America. We address both experimental measures of adaptation and the likelihood that it has escaped genetic constraints on adaptation imposed during its initial colonization.

### The hemlock woolly adelgid and cold tolerance

Hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), native to Asia, was introduced to eastern North America. It was first recorded in 1951 in Virginia (McClure, 1989; Suoto *et al.*, 1996) and reached Connecticut by 1985 (McClure, 1997). The current range (Fig. 1)



**Fig. 1.** Sample sites and range expansion of *Adelges tsugae* relative to the native range of eastern hemlock in North America. Map redrawn from USDA Forest Service and Little (1971).

of this insect is from North Carolina to coastal New Hampshire in the eastern United States, while the native range of hemlocks in this area is from Georgia to southern Canada (Godman and Lancaster, 1990). It feeds among the needles upon the parenchyma cells (Young *et al.*, 1995) of eastern hemlock (*Tsuga canadensis* Carriere) and Carolina hemlock (*Tsuga caroliniana* Englemann). It quickly reaches high densities, causing severe dieback and threatening whole stands (Orwig and Foster, 1998).

*Adelges tsugae* in eastern North America is parthenogenetic with an elaborate life cycle, described by McClure (1989). The wingless sistens generation overwinters on hemlock and, in New England, begins depositing egg sacs in late March. The eggs hatch into nymphs that develop into one of two morphs, the winged sexupara and wingless progrediens, at ratios that depend on population density (McClure, 1991). In Asia, where the species is a cyclic parthenogen, the sexuparae complete development on hemlock, then fly to spruce (*Picea* spp., the ancestral host in *Adelges*) to reproduce parthenogenetically. The resulting nymphs (the winged sexuales generation) mature on spruce and reproduce sexually, flying back to hemlock to oviposit. In North America, the sexupara–sexuales route is a genealogical dead end, failing because there are no native spruce species suitable for the sexuales nymphs (McClure, 1989). Meanwhile, the progrediens generation completes development on hemlock and begins parthenogenetic oviposition in June. These eggs hatch through July into crawler nymphs of the sistens generation. These find appropriate feeding sites on hemlock and enter aestivation (Salom *et al.*, 2001), then resume development in October and grow through

the winter (McClure, 1989). Dispersal occurs when crawler nymphs are carried by wind or phoretically on mammals and birds to new hemlock trees (McClure, 1990).

This life cycle requires the hemlock woolly adelgid to be exposed on twigs during the severest periods of winter. Parker *et al.* (1998, 1999; Skinner *et al.*, 2003) used cold-shock treatments to mimic winter minimum temperatures. They found that survivorship of *A. tsugae* decreases with the temperature and duration of the cold treatment, and also decreases for a given exposure level as the season progresses from February to March (Parker *et al.*, 1998, 1999). Exposure to sub-zero temperatures causes mortality by destroying cells found in adelgid haemolymph (Parker *et al.*, 2000). Parker *et al.* (1998) proposed that the spread of *A. tsugae* northward from its current distribution may be limited by its ability to survive average annual minimum temperatures below  $-20^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$ . Notably, these authors found that in all their cold treatments, there was always some survival, suggesting that sub-zero temperatures may select for cold tolerance in *A. tsugae* (Parker *et al.*, 1998). Skinner *et al.* (2003) found that adelgid populations from the northern and southern edges of the range had different tolerances to cold shock, in the direction predicted by adaptive change. However, environmental and maternal differences could not be ruled out, and such effects are implicated in the regulation of other aspects of *A. tsugae*'s life history (Salom *et al.*, 2001) as well as in cold-shock responses of other insects (Magiafoglou and Hoffmann, 2003).

To address the more general prediction that tolerance to environmental extremes should be evolutionarily labile in invasive species, we adopt a two-stage approach. First, we modelled genetic constraints imposed by mutation limitation and proposed a threshold population size above which mutation limitation should be negligible. Second, we tested the more proximate prediction of Parker *et al.* (1998) that the hemlock woolly adelgid may be evolving the ability to survive colder temperatures as it spreads northward from Virginia. We compared the cold-hardiness of *A. tsugae* from the southern and northern edges of its distribution in 2001, and used a common-garden experiment in 2002 to determine if differences were due to genetic changes.

### Evolutionary constraints imposed by mutation limitation

For invasive species, the ability to respond to selection should be constrained mainly by the availability of appropriate genotypes in the introduced genome. If the genome lacks such genotypes, because either the propagule experienced a severe bottleneck or the source population never had the requisite genetic variation, then evolution will be constrained by the availability of new mutants. However, with respect to the resistance to environmental extremes, it is reasonable to expect that the species in its native range would experience extremes of the same type, if not the same severity, as in the introduced range. In that case, a propagule of moderate size (say, 10–50 diploid individuals) may well contain genetic variation conferring at least partial resistance. Genetic variation in the native range is therefore of interest in predicting the evolutionary potential in the introduced range.

Where standing genetic variation is absent or insufficient in the propagule, the availability of new variants in the introduced range is influenced by the mutation rate and the population size. The average number of new mutants each generation is  $pNu$ , where  $p$  is the ploidy level,  $N$  is the (actual) population size and  $u$  is the mutation rate. How big must the population size be to provide sufficient new mutants to the evolving population? A complete answer is probabilistic: it depends on the genetic and physiological details of how the trait is expressed, which mutants happen to arise first, and the strength of selection (Elena and Lenski,

2003). One useful benchmark along this continuum of possibilities is the population size ( $N^*$ ) at which a new mutant arises at every base pair in the genome an average of once each generation, which is  $N^* = 1/(p u_G)$ , where  $u_G$  is the per-base mutation rate. Populations of this size will regularly generate favourable mutants and respond rapidly to selection. Only complex, epistatic and polygenic traits, or those requiring several base changes at a single locus, will show significant lag times before a response to selection commences. If mutants convey partial resistance, then recombination in sexual species will soon bring together favourable alleles and rapid evolution will occur even with multiple loci at this population size. Once an invasive species surpasses this population size, we should expect its evolution to be largely unconstrained. It is heuristically useful to cast  $N^*$  in terms of the geographic area  $A^*$  that holds this population, which we call the 'area of mutation saturation'. This area is  $A^* = N^*/D = 1/(p D u_G)$ , where the density of the invasive species is  $D$ . When  $A^*$  is substantively less than the entire range of an invading species, then that species will have escaped any evolutionary constraint imposed by mutation limitation.

While  $N^*$  is certainly a large number, it is by no means astronomical for most well-established, widespread invasive species with small body sizes. Such species are likely to have surpassed  $N^*$  relatively early in their invasions, and many will have areas of mutation saturation  $A^*$  that comprise relatively small parts of their geographic ranges. For example, in the Discussion we estimate  $A^*$  for the *A. tsugae* case from published entomological and forestry data, and find that the area within which mutation accumulation can constrain evolution is surprisingly small.

## METHODS

### Source-population experiment

Adelgids were collected from six sites, three in Maryland (Annapolis, Gaithersburg and Clarksburg) and three in western Massachusetts (Longmeadow, Holyoke and Amherst), in mid-January 2000. Winter temperature extremes from these areas are shown in Table 1. We collected 15 live, infested, hemlock twigs, each 15 cm in length, from each site. We counted the numbers of live sistens nymphs on each twig, and held the twigs for one month hydrated in florist's foam in a cool room at 2°C, separated to prevent cross-contamination. We randomly selected 10 twigs from each site, and exposed them to a cold snap for 36 h in a freezer at -15°C. (We chose this temperature regime because a previous attempt to cold-shock adelgids at -15°C for 72 h resulted in 100% mortality; by cutting the exposure time in half we hoped to reduce the mortality by roughly half.) After the adelgids were cold-shocked, we moved them back to 2°C. The five 'control' twigs stayed at 2°C for the duration of the experiment. After one week, we counted the numbers of living and dead sistens on each twig.

### Common-garden experiment

We controlled for environmental and maternal effects on hemlock woolly adelgid cold-hardiness by rearing offspring from Massachusetts and Maryland adelgids in a common garden for two generations. We used adelgids from the control twigs collected in January 2001 to rear *A. tsugae* offspring. These adelgids did not experience viability selection via the cold-shock treatment, so they were random samples of their source populations. Beginning

**Table 1.** Monthly low winter temperature extremes (°C), averaged over 1992–2002, from weather stations nearest to our study sites, and the corresponding sites

Weather station	Study site	Distance to site (miles)	December	January	February
Amherst, MA	Amherst, MA	5	−15.3 (1.1)	−20.1 (1.5)	−19.0 (1.4)
Amherst, MA	Holyoke, MA	18			
Windsor, CT	Longmeadow, MA	16.5	−13.4 (0.7)	−17.2 (1.0)	−16.7 (1.4)
Annapolis, MD	Annapolis, MD	5	−8.6 (0.6)	−10.6 (1.3)	−9.5 (1.3)
Clarksville, MD	Clarksville, MD	7	−13.1 (0.8)	−14.8 (1.0)	−14.0 (1.6)
Clarksville, MD	Gaithersburg, MD	20			

*Note:* Northern sites were significantly colder. Standard errors in parentheses.

in March 2001, these twigs were exposed to a thermal regime consisting of temperature increases from the storage temperature of 2°C to 7°C, then to 12°C, then 15°C at two-week intervals. Sistens on the twigs from Gaithersburg and Clarksburg in Maryland, and Longmeadow and Amherst in Massachusetts, successfully oviposited. In May 2001, each control twig was used to infest hemlock branches on five mildly infested, outwardly healthy trees in a common-garden plot near the Quabbin Reservoir in western Massachusetts. We distributed twigs from each source population onto separate branches of each tree to control for tree effects. These branches were inspected for sistens prior to the experimental treatment; none were found. We enclosed each branch for the season in a 30 cm<sup>2</sup> nylon mesh bag with a weave tight enough to trap the adelgids, but still allow for ventilation. Branches were collected in January 2002, and the second adelgid generation removed from the release population. We cut each branch into 20 twigs of 15 cm length and counted the numbers of live sistens on each twig. We randomly selected 15 twigs from each branch for the next cold-shock treatment, put them in a freezer at −15°C and, after 36 h, moved them back to 2°C. The five remaining ‘control’ twigs remained at 2°C. After one week, we counted the numbers of living sistens on each twig.

### Data analysis

We analysed survival differences between treatments (control vs. cold-shocked), between source regions (Maryland vs. Massachusetts), among sites (collecting localities) nested within source regions, and their interactions (treatment × region and treatment × site (region)), with each twig treated as a replicate. Site(region) and treatment × site(region) were treated as random effects. Logistic regression in this complex design yielded biased estimates of back-transformed survivorship proportions, showing especially severe biases for estimates of the treatment × site(region) parameters that were of greatest interest to us. We therefore computed arcsine-square root-transformed mean survivorship for each twig and used these in an analysis of variance (ANOVA), weighted by the sample size per twig. Back-transformed estimates of the marginal means from this model were close to the least-squares means of untransformed data, which are statistically unbiased. We report the unbiased least-squares means in the figures. All analyses were performed using JMP software (v. 5.0.1.2; SAS Institute, 1995).

To estimate the geographic area of mutation saturation,  $A^*$ , of hemlock woolly adelgids using density estimates from published entomological and forestry data, we require the ratio of twig surface area to leaf area of hemlock trees. We removed twigs from five healthy hemlock trees and sealed them in plastic bags for transport back to the laboratory. Needles were stripped from the twigs, placed on a flat-bed scanner in negligibly overlapping configurations and scanned at 600 dpi; the bare twigs were then scanned separately. The total area of each image was estimated using NIH Image (v1.63). The scanned twig area was multiplied by  $\pi$  to get the twig surface area, and the ratio of twig surface area to leaf area was determined for each plant sample.

## RESULTS

### 2001 source populations

We counted 5630 adelgids in this data set. There was sample-size variation among twigs, with  $\bar{n}_{\text{twig}} = 62.6 \pm 39.1$  sistens per twig (mean  $\pm$  standard deviation; range 7–182). The analysis of survivorship is summarized in Table 2. The cold-shock treatment had a strong effect, reducing overall survivorship to  $36.7 \pm 2.5\%$  from  $92.7 \pm 3.5\%$  in the controls (least-squares means and standard errors reported hereafter). We observed 12 of 30 control twigs with zero mortality, and one of 60 cold-shocked twigs had zero survivorship. Experiment-wide survivorship did not differ significantly among regions, or among sites within regions. However, there was a significant interaction between treatment and region ( $P = 0.044$ ), and for treatment among sites within regions ( $P = 0.035$ ), indicating that geographic variation exists in the response to cold shock. Massachusetts samples survived cold shock better than Maryland samples, even though Maryland samples had higher survivorship in the controls (Fig. 2a). Within Massachusetts, the Holyoke sample was much more sensitive to cold shock than the Amherst and Longmeadow samples; in Maryland, the Gaithersburg sample was much more sensitive than the Annapolis and Clarksburg samples (Fig. 2b).

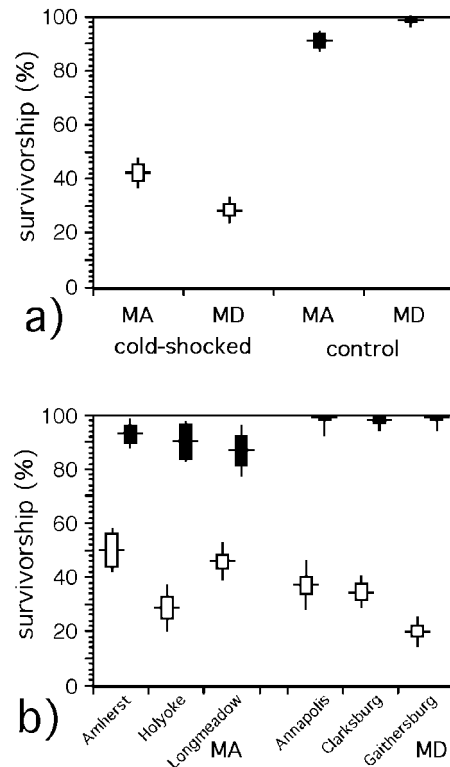
The significant interaction terms are consistent with the hypothesis that the adelgids are undergoing adaptive evolution in cold tolerance as they expand their range northward. Indeed, the coldest temperatures in winter, averaged monthly from 1992 to 2002 from weather stations near our study sites, were consistent with this pattern (Table 1). Our result

**Table 2.** Analysis of survivorship in the 2001 source populations, based on ANOVA of arcsine-square root-transformed survivorship proportions for individual twigs, weighted by the number of sistens per twig

Source	d.f.	SS	MS denom. synthesis	<i>F</i>	<i>P</i>
A. Treatment	1	569.447	0.8791E + 0.1209F	205.338	~0
B. Region	1	1.385	0.8791E + 0.1209F	0.562	0.491
C. Site(region)	4	10.607	E	0.883	0.547
D. Treatment $\times$ region	1	21.630	0.8791E + 0.1209F	7.799	0.044
E. Treatment $\times$ site(region)	4	12.013	F	2.723	0.035
F. Residual	78	86.030			

*Note:* Site(region) and treatment  $\times$  site(region) were treated as random effects.  $N = 5633$  sistens counted as dead or alive.





**Fig. 2.** Percent survival of *Adelges tsugae* collected in January 2001 from Maryland and Massachusetts and exposed to  $-15^{\circ}\text{C}$  (cold-shocked: ■) or  $2^{\circ}\text{C}$  (control: □). (a) Treatment  $\times$  region effect. (b) Treatment  $\times$  site(region) effect.

agrees with Skinner *et al.* (2003), who used a similar experimental design. However, since these adelgids were taken directly from the field, these outcomes can also be explained by environmental and maternal-effect differences. For example, the phenologies of these populations differ, possibly putting them in different stages of development and causing variation in sensitivity to cold shock. We therefore reared the offspring of adelgids from the control twigs in common environments in the Amherst region and reanalysed them the following year.

### 2002 common-garden populations

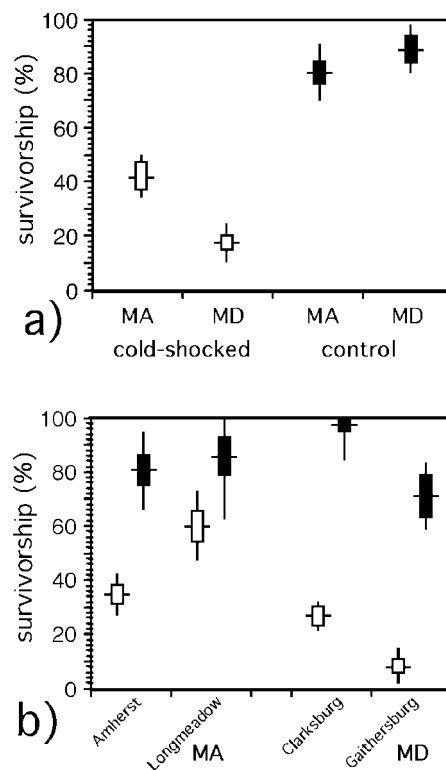
Altogether, 1753 living and dead adelgids were counted among 81 twigs in the common-garden data set, with  $\bar{n}_{\text{twig}} = 21.7 \pm 19.6$  sistens per twig (mean  $\pm$  standard deviation; range 2–123). Table 3 shows the analysis of survivorship. Again the cold-shock treatment sharply reduced survivorship to  $32.7 \pm 2.6\%$  from  $83.6 \pm 3.6\%$  in the controls. We observed 12 of 26 control twigs with zero mortality; 6 of 55 cold-shocked twigs had zero survivorship and one treated twig (holding four sistens) had zero mortality. We again found no differences in overall survivorship among regions, but differences did arise among sites within regions. This effect was traced to the Gaithersburg population, which showed lower average

survivorship ( $39.9 \pm 3.6\%$ ) than the other populations (range  $57.8 \pm 4.2\%$  to  $72.9 \pm 7.0\%$ ). The significant treatment  $\times$  region interaction seen in the 2001 source populations was somewhat more pronounced in the common-garden experiment: mortality due to cold shock was significantly higher in offspring from the Maryland samples ( $P = 0.01$ ; Fig. 3).

**Table 3.** Analysis of survivorship in the 2002 common-garden experiment, based on ANOVA of arcsine-square root-transformed survivorship proportions for individual twigs, weighted by the number of sistens per twig

Source	d.f.	SS	MS	denom. synthesis	<i>F</i>	<i>P</i>
A. Treatment	1	125.125	0.7898E + 0.2102F		189.532	~0
B. Region	1	6.197	0.7898E + 0.2102F		0.514	0.546
C. Site(region)	2	29.994	E		26.396	0.037
D. Treatment $\times$ region	1	12.804	0.7898E + 0.2102F		19.395	0.010
E. Treatment $\times$ site(region)	2	1.136	F		0.565	0.571
F. Residual	73	73.438				

*Note:* Site(region) and treatment  $\times$  site(region) were treated as random effects.  $N = 1753$  sistens counted as dead or alive.



**Fig. 3.** Percent survival of *Adelges tsugae* by source population, reared in the common garden and exposed to  $-15^{\circ}\text{C}$  (cold-shocked: ■) or  $2^{\circ}\text{C}$  (control: □). (a) Treatment  $\times$  region effect. (b) Treatment  $\times$  site(region) effect (not significant).

The treatment  $\times$  site(region) interaction was not significant in this experiment; we were unable to detect meaningful among-site variation in sensitivity to cold shock. This strong shift in significance between experiments, from  $P = 0.035$  to  $P = 0.571$ , suggests that these site effects in the source populations may have been due to initial environmental or maternal differences that we removed in the common-garden experiment.

### Estimation of $A^*$ from published data

How big must adelgid population density be to overcome the limiting effects of mutation, and what geographic area would this population size occupy? We can gain a rough perspective on the extent of mutation limitation by estimating the area of mutation saturation,  $A^* = 1/(2D\mu_G)$ . Our approach, philosophically rooted in risk analysis, is to conservatively estimate the upper bound of  $A^*$ , which measures the maximum extent that mutation limitation can play, because precise values of the genomic mutation rate  $\mu_G$  are unavailable. We obtain the adelgid density from published values using  $D = D_{\text{twig}} f A_L(A_B) h$ , where  $D_{\text{twig}}$  is the adelgid density on twigs,  $f$  is the ratio of twig surface area to leaf area,  $A_L(A_B)$  is the leaf surface area as a function of the basal area  $A_B$  (trunk cross-sectional area at breast height), and  $h$  is the fraction of forest that is in hemlock stands. McClure (1991) reported densities of  $D_{\text{twig}} = 0.1\text{--}2.0$  adelgids  $\cdot \text{mm}^{-2}$  on the twigs of infested trees, with  $>0.2$  adelgids  $\cdot \text{mm}^{-2}$  precluding new hemlock growth. We measured the ratio of hemlock twig surface area to leaf area in this study to be  $f = 0.35 \pm 0.01$  (mean  $\pm$  standard error). Kenefic and Seymour (1999) provided a regression for total leaf area against the basal area for hemlock,  $A_L(A_B) = 10.8264 + 0.1454 \times A_B$ , with  $A_B$  in  $\text{cm}^2$  and  $A_L$  in  $\text{m}^2$ . Orwig and Foster (1998) provided mean basal area measures for stands of  $A_B = 19\text{--}75$   $\text{m}^2 \cdot \text{ha}^{-1}$  depending on stand age and reported that 90% of stands were infested. They also gave numbers allowing us to calculate that  $h = 3.4\%$  in Connecticut (including forested and urbanized areas), whereas Royle and Lathrop (1997) reported that hemlock stands occupy about  $h = 6\%$  of the landscape in western New Jersey. From these values we obtain densities of  $D = 1.3 \times 10^8$  to  $2.2 \times 10^9$  adelgids  $\cdot \text{ha}^{-1}$  in forests where all trees are infested. For per-base mutation rates as (conservatively) low as  $\mu_G \approx 10^{-10}$ , and conservative values from the above ranges for all the other measures, enough mutants to saturate the adelgid genome would still arise each generation within an area of  $A^* = 1/(2D\mu_G) \approx 37$  ha. This area comprises a very small part of the expanding range of *A. tsugae*. At more reasonable rates of  $\mu_G \approx 10^{-9}$  and higher average infestation densities of  $D_{\text{twig}} = 0.5$  adelgids  $\cdot \text{mm}^{-2}$  (McClure, 1991), a forested area of only  $A^* < 1$  ha is required for mutation saturation. With two generations annually, these rough numbers suggest that most hemlock stands will easily generate sufficient mutants for adaptive evolutionary change in their adelgid populations within the first few years of infestation.

### DISCUSSION

The *Adelges tsugae* example supports the prediction that invasive species should show evidence of adaptation as they expand their ranges. In particular, populations of many invading species should have risen well beyond the size needed to escape evolutionary constraints imposed by mutation limitation, and the susceptibility of invasive species to new patterns of environmental extremes should impose strong evolutionary pressure. Population densities indicate that hemlock woolly adelgids, *A. tsugae*, should generate

tremendous genetic variation within very small geographic areas. The significant treatment  $\times$  region interaction indicates that cold-tolerance in the hemlock woolly adelgid has evolved adaptively in little more than 100 generations as this introduced forest pest has expanded its range northward from Virginia to Massachusetts. The common-garden experiment eliminated the possibility that the interaction was caused by differential environmental cues, acting directly (e.g. Parker *et al.*, 2003) or via maternal effects (e.g. Magiafoglou and Hoffmann, 2003), since the adelgids were reared side by side through the progrediens and sistens generations prior to treatment.

### Evolutionary genetics of the *Adelges tsugae* invasion

*Adelges tsugae* is adapting to severe cold conditions as it expands its range northward. It therefore clearly sustains sufficient genetic variation in its eastern North American populations for adaptive evolution, even though it probably was introduced as a small, horticulturally associated propagule in Richmond, Virginia, before 1950 (Suoto *et al.*, 1996). Even so, our results shed little light on the founding population size or amount of standing genetic diversity in the original propagule. This is partly because we do not yet know the extent of genetic variation in the native source populations, so we do not know the probabilities of capturing sufficient variation in propagules of different sizes. However, regardless of the initial variation, the very small area of mutation saturation,  $A^*$ , in *A. tsugae* indicates that its adaptation in eastern North America is currently not limited by the availability of new mutations.

Though mutation does not seem to be limiting, the lack of recombination in the eastern North American hemlock woolly adelgid population associated with its obligate parthenogenesis should sharply constrain its evolutionary rate relative to sexual species. Because of diploidy, new mutants mainly arise as heterozygotes and are only expressed if the phenotype is dominant. Recessive homozygotes of new mutants would be extremely rare without sexual recombination. Polygenic adaptations in these adelgids therefore mainly arise by successive steps in an asexual-cladogenic process, with each mutant needing to be dominant and advantageous in its own right in order to rise to sufficient frequency in the spreading population that it has a chance to be hit with the next favourable mutation. More rarely, depending ultimately on the rate that adaptive alleles spread during the invasion process, a neutral, recessive or mildly deleterious allele may be swept along to high frequency in disequilibrium with an advantageous allele at an unrelated locus, then hit by a second mutation that confers an epistatic advantage. Also rarely, gene conversion may generate recessive homozygotes from heterozygotes, a process implicated in *Drosophila melanogaster*'s range expansion (Andolfatto and Wall, 2003). The low frequencies of these events constrain the efficiency of adaptation relative to outcrossing species, requiring a much greater mutation rate or population size to permit steady adaptive change in the face of directional selection. A corollary is that genetic variation might well have been limiting in the original Virginia propagule, but constrained by the lack of recombination rather than a paucity of available allelic diversity.

The lack of recombination in the hemlock woolly adelgid also blurs the distinction between additive and non-additive genetic variance when considering its evolutionary potential. In outcrossing species, the rate of adaptive evolution depends only on the additive portion of genetic variation in the population (Lynch and Walsh, 1998), because the non-additive influences on the phenotype, comprised of dominant, epistatic, genotype  $\times$  environment,

and covariance effects, are broken up each generation by recombination in the offspring. In parthenogens, epistatic and dominance effects are passed on to offspring, so most of the non-additive genetic variance does contribute to evolutionary change. Constraints due to inbreeding depression are also eliminated in parthenogenetic systems. These changes ameliorate the genetic constraint on adaptation somewhat, but they cannot make up for the loss of recombinatorial power that accompanies parthenogenesis.

### Evolution in other invasions

Though numerous examples of adaptations to abiotic extremes exist that *preceded* range expansion (e.g. Lee, 1999; Lee and Petersen, 2002), few besides the hemlock woolly adelgid example here have been shown to have occurred *during the course of* a biotic invasion. Thistles in the genus *Onopordum* underwent extensive hybridization and introgression associated with their invasion of Australia (O'Hanlon *et al.*, 1999), likely involving some degree of adaptation to dry conditions. There, genetic variance in the invader is presumed to have been augmented by hybridization. Abiotic extremes aside, Siemann and Rogers (2001) documented evolutionary changes that probably occurred during the course of the invasion of North America by the Chinese tallow tree, *Sapium sebiferum*. In a common-garden experiment, genotypes further from the likely introduction site showed traits associated with increased competitive ability and reduced resistance to herbivory. It is not yet clear whether this is due to changes in the spectrum of competitors and natural enemies, as suggested by Siemann and Rogers (2001), or traits associated with increased dispersal at the leading edge of the range expansion front (cf. Travis and Dytham, 2002; Simmons and Thomas, 2004); both could be involved. Similarly, St. John's wort (*Hypericum perforatum*), invasive in North America, has evolved clines in several traits that parallel clines in its native range, and genetic markers identifying source populations rule out explanations that the founding propagules were pre-adapted (Maron *et al.*, 2004). Inasmuch as adaptation is quite likely to be an important determinant of range expansion (Lambrinos, 2004), there remains a distinct need to document more examples of genetic changes near the expanding front of an invasion (Lee, 2002).

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