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Altered genotypic and phenotypic frequencies of aphid populations under enriched CO_2 and O_3 atmospheres

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Abstract

Environmental change is anticipated to negatively affect both plant and animal populations. As abiotic factors rapidly change habitat suitability, projections range from altered genetic diversity to wide-spread species loss. Here, we assess the degree to which changes in atmospheric composition associated with environmental change will influence not only the abundance, but also the genotypic/phenotypic diversity, of herbivore populations. Using free-air CO_2 and O_3 enrichment (FACE) technology, we assess numerical responses of pea aphids (*Acyrthosiphon pisum*) exhibiting a pink–green genetic polymorphism and an environmentally determined wing polyphenism on broad bean plants (*Vicia faba*) under enriched CO_2 and/or O_3 atmospheres, over multiple generations. We show that these two greenhouse gases alter not only aphid population sizes, but also genotypic and phenotypic frequencies. As the green genotype was positively influenced by elevated CO_2 levels, but the pink genotype was not, genotypic frequencies (pink morph : green morph) ranged from 1 : 1 to 9 : 1. These two genotypes also displayed marked differences in phenotypic frequencies. The pink genotype exhibited higher levels of wing induction under all atmospheric treatments, however, this polyphenism was negatively influenced by elevated O_3 levels. Resultantly, frequencies of winged phenotypes (pink morph : green morph) varied from 10 : 1 to 332 : 1. Thus, atmospheric conditions associated with environmental change may alter not just overall population sizes, but also genotypic and phenotypic frequencies of herbivore populations, thereby influencing community and ecosystem functioning.

Keywords: air pollution, carbon dioxide, climate change, plasticity, polymorphism, polyphenism, ozone

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Introduction

Serious concerns have been raised about the effects of global environmental change on community and ecosystem functioning (Körner & Bazzaz, 1996; Niklaus *et al*., 2001). Because of the rapidity of environmental change, plant and animal populations may be subject to intense selection pressures (Vitousek, 1994). Altered abiotic factors, such as increased temperature or changes in precipitation, may cause many species' habitats to shift geographically, with present territories often becoming uninhabitable (Gjerdrum *et al*., 2003; Inouye *et al*., 2003; Root *et al*., 2003). It is unclear, however, whether environmental change will induce extinction events (Williams *et al*., 2003; Thomas *et al*., 2004) or if plant and animal populations will persist, although with altered diversity (Bazzaz *et al*., 1995; Andalo *et al*., 2001; Tilman & Lehman, 2001).

Greenhouse gases are key factors associated with global environmental change (IPCC, 2001). Two of these gases, CO_2 and tropospheric O_3, are generally stimulatory and inhibitory, respectively, for plant growth processes (Saxe *et al*., 1998; Ceulemans *et al*., 1999; Karnosky *et al*., 2003). The effects of these two gases, however, may offset each other to some degree (Dickson *et al*., 1998; Heagle *et al*., 1998; Donnelly *et al*., 2000). Elevated concentrations of CO_2 and O_3 also alter the performance of organisms, such as insect herbivores, at higher trophic levels (Percy *et al*., 2002; Stacey &

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Fellowes, 2002). Lepidoptera generally increase feeding rates on nutrient diluted, CO_2-enriched foliage, resulting in little or no change in growth (Lindroth, 1996; Bezemer & Jones, 1998; Coviella & Trumble, 1999). Meanwhile, performance of the same insects may improve on vegetation grown under elevated O_3 (Kopper & Lindroth, 2003). Phloem-feeding insects, such as aphids, however, exhibit equivocal responses such as increased, decreased, and no change in growth and offspring production in response to plants grown under elevated CO_2 or O_3 (Hughes & Bazzaz, 2001; Holopainen, 2002).

Scaling short-term developmental responses of individual insects to population-level effects has been relatively unsuccessful (Bezemer *et al*., 1999; Awmack *et al*., 2004), as reproductive and somatic tissue allocation may be altered in a non-linear fashion in response to various environmental factors (Leather, 1988). Environmental change also has the potential to alter not only overall herbivore population sizes, but genotypic and phenotypic frequencies (Thomas *et al*., 2001; Rank & Dahlhoff, 2002). By evaluating genotypic and phenotypic frequencies (i.e. the proportion of each genotype and phenotype out of the total population of genotypes and phenotypes; Conner & Hartl, 2004), it is possible to determine the direction and rate of selection on a trait in a population. Moreover, as herbivore genotypic and phenotypic frequencies oscillate, higher trophic levels are likely to be affected. For example, the top-down effects of natural enemies on prey populations are strongly influenced by the frequencies of genotypes, and associated patterns of phenotypic expression, in herbivore populations (Lewontin, 1970; Losey *et al*., 1997).

Aphids are good model organisms with which to assess the effects of environmental change. As aphids have short generation times, experiments can be conducted over multiple generations (Dixon, 1998). Furthermore, as aphids are parthenogenetic during the summer months, genetic colour polymorphisms can be used as genotypic markers to identify individual asexual lineages (Tomiuk & Wöhrmann, 1982; Conner & Hartl, 2004). Pea aphids, *Acyrthosiphon pisum*, for example, exhibit a pink–green genetic polymorphism (Losey *et al*., 1997). Besides colour, other traits differ among asexual lineages, such as the ability to exhibit an environmentally determined wing induction response (Müller *et al*., 2001). The extent to which this wing polyphenism is induced by atmospheric change may have large effects on trophic functioning. Winged individuals evade natural enemies more easily (Dixon, 1998) and are chief plant virus vectors (Ng & Perry, 2004).

Here, we describe a field experiment, conducted at the Aspen free-air CO_2 and O_3 enrichment (FACE) site, investigating the effects of elevated levels of CO_2 and O_3 on the genotypic and phenotypic frequencies of pea aphid populations. Firstly, we assessed whether atmospheric change significantly altered the frequencies of individuals exhibiting a pink–green genetic polymorphism, as evidenced by different numbers of the two morphs. Secondly, we assessed whether atmospheric composition altered phenotypic frequencies (i.e. the proportion of individuals of the two genotypes exhibiting an environmentally determined wing polyphenism).

Methods

FACE experiment

Aspen FACE is a 32 ha site located near Rhinelander, WI, USA (45.7° latitude, 89.7° longitude), consisting of 12, 30 m diameter rings. The site was constructed and trees planted inside each ring in 1997 (Dickson *et al*., 2000). Each FACE ring is divided into three sectors: (1) trembling aspen genotypes (*Populus tremuloides* Michx.); (2) trembling aspen and sugar maple (*Acer saccharum* Marsh.); and (3) trembling aspen and paper birch (*Betula papyrifera* Marsh.). Stands have been exposed to ambient or elevated levels of CO_2 and/or O_3 since 1998 (Dickson *et al*., 2000).

The FACE experiment is a randomized complete block design, consisting of three blocks of four rings (i.e. treatments): (1) control ($367 \pm 15\,\mu L\,L^{-1}$ CO_2 and $38 \pm 13\,nL\,L^{-1}$ O_3), (2) elevated CO_2 ($+CO_2$, $537 \pm 77\,\mu L\,L^{-1}$), (3) elevated O_3 ($+O_3$, $51 \pm 22\,nL\,L^{-1}$), and (4) elevated CO_2 and O_3 ($+CO_2+O_3$, $537 \pm 77\,\mu L\,L^{-1}$ $+ 51 \pm 22\,nL\,L^{-1}$, respectively) (Dickson *et al*., 2000). Carbon dioxide levels are elevated to represent levels predicted for 2060, while ozone levels are elevated in a diurnal pattern approximately 1.5-fold that of ambient levels (Dickson *et al*., 2000). A trace-gas monitoring system continually adjusts the concentrations of CO_2 and O_3 applied to the forest stands.

Changes in herbivore genotypic and phenotypic frequencies

Fifteen, 15 cm diameter pots were filled with local topsoil (Musson Brothers, Rhinelander, WI, USA) and placed within the aspen-maple sector of each of the 12 FACE rings. Two broad bean, *Vicia faba* cv. Broad Windsor, seeds were planted in each pot. A wooden stake was placed mid-centre and a $30 \times 60\,cm^2$ mesh bag was placed over each pot and attached with rubber bands, to deny access to naturally colonizing insects. After the plants had germinated and reached a height of ca. 45 cm, 8–10 leaf pairs (averaged across FACE rings)

insects were added to treatment pots. Each pot was randomly assigned, using a random number generator (JMP IN 5.1, SAS Institute, 2005) to one of the following treatments: (1) no aphids (control), (2) two green apterous adult pea aphids, or (3) two pink apterous adult pea aphids ($n = 3$, 6, and 6 of each treatment per ring). Genotypes were reared in different pots, to better understand the sole effect of atmospheric composition on aphid population dynamics, without the confounding effect of intraspecific competition. We used the pea aphid as a model herbivore species as it naturally colonizes understory plants at Aspen FACE.

Experimental aphids were derived from single green and pink asexual lineages, initially obtained from red clover, *Trifolium pratense*, in Rhinelander, WI. The two genotypes had been reared in the laboratory for approximately 12 months prior to the experiment. Aphids used in the experiment were newly moulted adults reared under ambient, low-density conditions, to minimize any prior effects of crowding on winged offspring production. After placement of aphids on the treatment plants, five pots from each FACE ring ($n = 1$, 2, and 2 of the aforementioned treatments, respectively) were destructively sampled each week, for 3 weeks (ca. two aphid generations). We chose to run our experiment for only 3 weeks so that declining host plant quality, as a result of feeding damage from large aphid populations, would not be a factor in our experiment. Each week, plants were thoroughly examined for numbers, ages (first/second instars (ca. 0–2 mm), third/fourth instars (ca. 2–4 mm) and adults (>4 mm)), and if adult, phenotypes (unwinged and winged), of aphids developing under the different atmospheres.

Statistics

Data were analysed with split-plot ANOVAs (JMP IN 5.1, SAS Institute, 2005). Whole-plot effects consisted of the atmospheric treatments CO_2 (ambient vs. elevated) and O_3 (ambient vs. elevated), fully crossed. Ring block was incorporated as a whole-plot random variable (block 1 vs. 2 vs. 3). Subplot factors were time (week 1 vs. 2 vs. 3) and aphid genotype (pink vs. green). All interactions among gas treatments, time, and genotype were included as subplot interactions. A separate split-plot ANOVA was performed for each aphid age class and phenotype (i.e., first/second instars, third/fourth instars, unwinged adults, and winged adults).

As experimental replication of the fumigation treatments involves entire FACE rings, individual assays of each genotype within each ring are not true replicates. As a result, numbers of aphids of each age group, from each treatment pot, were calculated. We then obtained the mean number of aphids of each age class and phenotype, per genotype per FACE ring per week. Numbers of aphids were transformed $[x' = \sqrt{(x)} + \sqrt{(x+1)}]$ prior to analysis to achieve normality and equalize variances (Zar, 1984). Our no aphid (control)

Table 1 The effects of CO_2 and/or O_3 on the abundances of different age classes and phenotypes of single genotypes of pink and green pea aphids, *Acyrthosiphon pisum*, on broad bean, *Vicia faba*, as determined by split-plot ANOVAs

	First/second instars		Third/fourth instars		Apterous adults		Alate adults	
Treatment$_{(df)}$	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
$CO_{2\ (1,8)}$	7.81	**0.02**	3.57	0.09	10.58	**0.008**	0.05	0.82
$O_{3\ (1,8)}$	0.01	0.97	2.05	0.18	0.02	0.90	5.51	**0.04**
$CO_2 \times O_{3\ (1,8)}$	4.79	**0.05**	1.54	0.24	8.54	**0.01**	0.08	0.78
$Time_{(2,34)}$	53.86	**<0.001**	34.81	**<0.001**	60.48	**<0.001**	22.85	**<0.001**
$CO_2 \times time_{\ (2,34)}$	4.90	**0.01**	1.38	0.26	3.22	**0.05**	0.38	0.68
$O_3 \times time_{\ (2,34)}$	0.45	0.64	0.69	0.51	0.43	0.65	3.61	**0.04**
$CO_2 \times O_3 \times time_{(2,34)}$	2.93	0.07	1.08	0.35	2.16	0.13	0.07	0.94
$Genotype_{(1,34)}$	19.09	**<0.001**	12.87	**<0.001**	11.80	**0.002**	40.91	**<0.001**
$CO_2 \times genotype_{(1,34)}$	6.14	**0.02**	5.71	**0.02**	4.35	**0.04**	0.38	0.28
$O_3 \times genotype_{(1,34)}$	0.12	0.74	0.38	0.54	0.12	0.73	5.35	**0.03**
$CO_2 \times O_3 \times genotype_{(1,34)}$	2.01	0.17	1.23	0.28	2.32	0.14	0.07	0.79
$Time \times genotype_{(1,34)}$	7.82	**0.002**	0.19	0.83	7.50	**0.002**	16.64	**<0.001**
$CO_2 \times time \times genotype_{(1,34)}$	2.74	0.08	1.18	0.32	1.05	0.36	1.76	0.19
$O_3 \times time \times genotype_{(1,34)}$	0.10	0.91	0.29	0.75	0.06	0.95	1.48	0.24
$CO_2 \times O_3 \times time \times genotype_{(1,34)}$	2.79	0.08	0.91	0.41	3.57	**0.04**	0.12	0.89

Bold values indicate significant ($P \leq 0.05$) effects.

pots were not included in the statistical analyses, but rather, allowed us to verify that natural aphid populations were not colonizing our experimental plants.

Results

We observed significant differences in the numerical responses of different aphid genotypes to altered atmospheric conditions (Table 1). The pink genotype showed a high rate of population growth under all atmospheres, while atmospheric composition strongly influenced the population sizes of the green genotype (Fig. 1). The pink genotype markedly outperformed the green genotype, largely because of strong genotype × CO_2 interactions (Fig. 1). The green genotype exhibited increased population growth, reaching levels similar to that of the pink genotype, when both CO_2 and O_3 were elevated. In general, these differences in population sizes became more pronounced over time. As a result, the genotypic frequencies ranged from 1 : 1 to 9 : 1 (pink morph : green morph), depending on the atmospheric composition (Fig. 2).

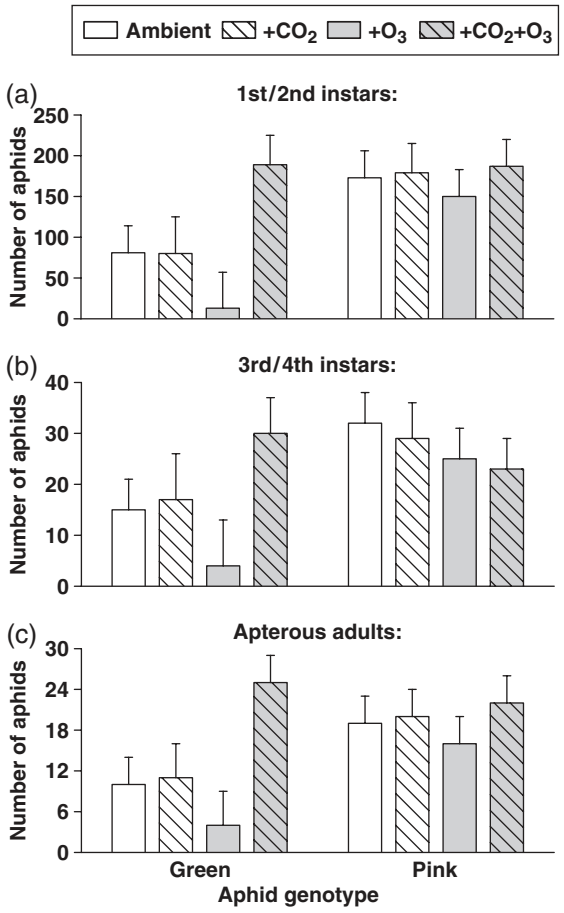

Fig. 1 Numbers (mean + 1 SE) of pink and green pea aphids, of each age class, under all atmospheric treatments (ambient, + CO_2, + O_3, and + CO_2 + O_3). See Table 1 for statistical significance.

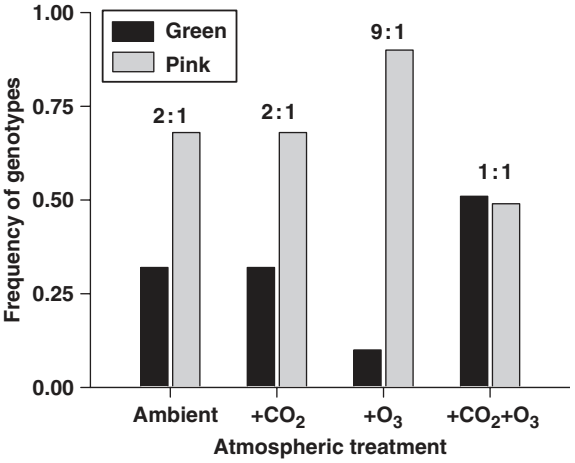

Fig. 2 Genotypic frequencies of pea aphid populations under different atmospheric treatments. All age classes have been combined, excluding winged adults. Ratios above columns represent the genotypic frequencies (pink morph : green morph) of the aphid populations.

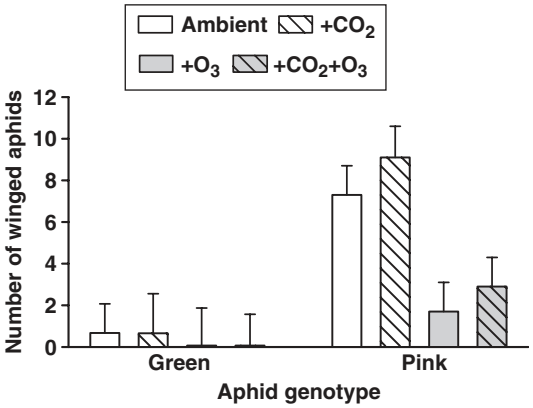

Fig. 3 Numbers (mean + 1 SE) of pink and green pea aphids, exhibiting wing-induction responses, under all atmospheric treatments (ambient, + CO_2, + O_3, and + CO_2 + O_3). See Table 1 for statistical significance.

Atmospheric conditions also influenced phenotypic expression of the two genotypes. Unlike genotypic frequencies, which were largely CO_2 dependent, wing induction responses were strongly O_3 dependent (Table 1). When O_3 levels were elevated, wing induction responses were depressed and became even more so over the duration of the experiment (Table 1, Fig. 3). The pink genotype was much more likely to exhibit wing induction, but elevated O_3 levels, both singly and in combination with elevated CO_2, depressed wing-induction responses more strongly in the pink than the green genotype (Fig. 3). As a result, frequencies of adult

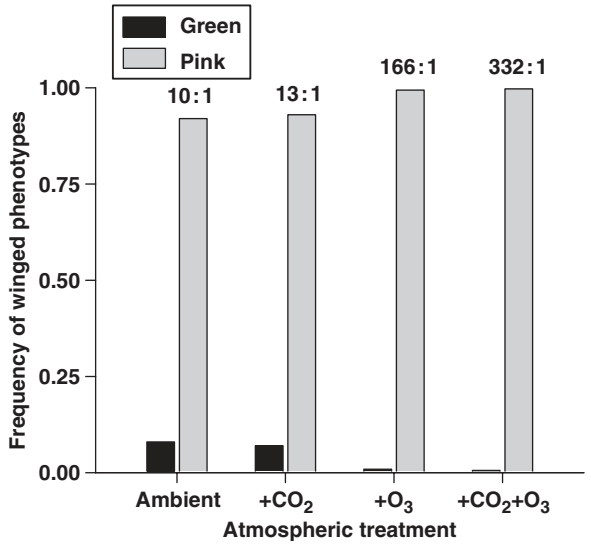

Fig. 4 Phenotypic frequencies (i.e., the number of individuals exhibiting a wing-induction polyphenism), of pea aphid populations under different atmospheric treatments. Ratios above columns represent the frequencies of winged phenotypes (pink morph : green morph) of the aphid populations.

aphids exhibiting wing-induction responses ranged from 10 : 1 to 332 : 1 (pink morph : green morph) (Fig. 4). The latter, more heavily skewed, phenotypic frequencies are based on very small values. It was clear, however, that wing-induction responses were exhibited almost exclusively by the pink genotype across all gas treatments.

Discussion

Environmental change has the potential to alter the composition, and hence stability, of communities and ecosystems (McNaughton, 1993; Tilman & Downing, 1994; Vitousek, 1994; Tilman, 1999). The success or failure of taxa in future environments will depend to a large degree on the amount of genetic, and associated phenotypic, diversity within populations and whether genotypes can cope with shifting selection pressures (Potvin & Tousignant, 1997; Etterson, 2004). Here, we have shown that elevated levels of CO_2 and tropospheric O_3, associated with environmental change, alter both the genotypic and phenotypic frequencies of aphid populations. As changes in both genotypic and phenotypic frequencies of aphid populations were observed in only two generations, our FACE experiment provides confirmatory evidence that some genotypes are relatively impervious to, while other genotypes are strongly influenced by, atmospheric composition.

Numerical responses of aphid populations, and resulting genotypic frequencies, were chiefly influenced by CO_2 levels. The green genotype responded favorably to enriched CO_2 atmospheres, primarily when O_3 was also elevated. Our pink genotype, however, did not exhibit substantial population changes in response to either CO_2 or O_3. The effects of these two greenhouse gases on population growth were consistent across instars for both genotypes, indicating that the gases do not differentially alter the growth and development of juveniles vs. adults, as is true for some aphid species (Awmack *et al*., 1997). Perhaps most interestingly, these data suggest that increases, decreases, and no change in aphid population sizes may all be possible outcomes in response to altered atmospheric conditions (Bezemer *et al*., 1999; Hughes & Bazzaz, 2001; Holopainen, 2002), depending on the herbivore genotypes used in experiments. Unfortunately, the vast majority of experiments on aphids and other herbivores do not specify the lineages of the insects.

Phenotypic frequencies of aphid populations (i.e., wing-induction responses) were also altered in response to atmospheric composition. Our pink genotype, compared with our green genotype, exhibited much higher rates of phenotypic plasticity. While CO_2 levels were more predictive of overall population sizes, aphid polyphenisms were more dependent on O_3 levels. Whether the costs of producing winged progeny under enriched O_3 atmospheres are prohibitive is uncertain (Mondor *et al*., 2004). An alternative explanation is that plants grown under enriched O_3 atmospheres are of higher nutritional quality (Awmack *et al*., unpublished data), thus reducing wing-induction responses in offspring. Irrespective of the underlying mechanism, the number of winged morphs in a colony and the factors inducing these phenotypic changes (Müller *et al*., 2001) are of great interest, as large numbers of winged morphs could greatly alter plant-virus dynamics (Werker *et al*., 1998).

It is intriguing that the genotype most affected by atmospheric conditions exhibited low levels of phenotypic plasticity, at least with respect to wing-induction responses. Meanwhile, the genotype little affected by enriched CO_2 and O_3 environments exhibited high levels of plasticity. Widely fluctuating environmental conditions are believed to be one consequence of climate change (Schneider, 1993). Furthermore, phenotypic plasticity is believed, in some instances, to facilitate genetic adaptation of organisms to novel environments (Sakai *et al*., 2001; Price *et al*., 2003; Yeh & Price, 2004). It is tempting to hypothesize that genotypes with greater levels of phenotypic plasticity will be better equipped to cope with changing climatic conditions (Thomas *et al*., 2001). The relationship between plasticity and environmental tolerance, however, will undoubtedly depend on what trait is being considered. As environmental

change is occurring at an unprecedented rate (Vitousek, 1994), it is difficult to predict, *a priori*, whether altered phenotypic expression under conditions of atmospheric change is adaptive (Mondor *et al*., 2004). Such reaction norms (Schlichting & Smith, 2002) will undoubtedly be the subject of increased investigation as climate change progresses.

In conclusion, our FACE experiment demonstrates that idiosyncratic life history responses of herbivores to global atmospheric change may not be only species-specific, but genotype-specific. A large degree of intraspecific variation may explain why aphid life history responses to CO_2 and O_3 have been so variable in previous research (Bezemer *et al*., 1999; Hughes & Bazzaz, 2001; Holopainen, 2002). Such intraspecific heterogeneity may cascade among trophic levels, altering community and ecosystem functioning (Whitham *et al*., 2003).

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