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Detecting small-scale genotype–environment interactions in apomictic dandelion (*Taraxacum officinale*) populations

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Abstract

Studies of genotype × environment interactions (G × E) and local adaptation provide critical tests of natural selection's ability to counter opposing forces such as gene flow. Such studies may be greatly facilitated in asexual species, given the possibility for experimental replication at the level of true genotypes (rather than populations) and the possibility of using molecular markers to assess genotype–environment associations in the field (neither of which is possible for most sexual species). Here, we tested for G × E in asexual dandelions (*Taraxacum officinale*) by subjecting six genotypes to experimental drought, mown and benign (control) conditions and subsequently using microsatellites to assess genotype–environment associations in the field. We found strong G × E, with genotypes that performed poorly under benign conditions showing the highest performance under stressful conditions (drought or mown). Our six focal genotypes comprise > 80% of plants in local populations. The most common genotype in the field showed its highest relative performance under mown conditions (the most common habitat in our study area), and almost all plants of this genotype in the field were found growing in mowed lawns. Genotypes performing best under benign experimental conditions were found most frequently in unmown conditions in the field. These results are strongly indicative of local adaptation at a very small scale, with unmown microsites of only a few square metres typically embedded within larger mown lawns. By studying an asexual species, we were able to map genotypes with known ecological characteristics to environments with high spatial precision.

Introduction

How genetic variation is maintained in natural populations is one of the most enduring questions in evolutionary biology (Lewontin, 1974; Kimura, 1983; Gillespie, 1991). The maintenance of genetic variation in asexual populations has been of particular interest because, without recombination, directional selection – which is frequently observed in natural populations (Kingsolver *et al.*, 2001) – is expected to eliminate genetic variation across the entire genome simultaneously (Sebens & Thorne, 1985). Although studies have addressed the origins and maintenance of genetic diversity in asexual populations (e.g. Rainey & Travisano, 1998; Weeks & Hoffman, 2008), few have explicitly explored the role of genotype × environment interactions, especially under natural field conditions (but see Vavrek, 1998; Stratton, 1994, 1995; Pantel *et al.*, 2011).

Genotype × environment interactions for fitness (G × E) are said to occur when the relative fitness of different genotypes depends on local environmental conditions, resulting in divergent selection (Kawecki & Ebert, 2004). G × E might lead to local adaptation, whereby the genotypes found in a particular environment are those with greatest fitness in that environment, but

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this can be prevented or weakened by genetic drift, temporal environmental variability and particularly gene flow (Lenormand, 2002; Kawecki & Ebert, 2004; Saether *et al.*, 2007; Leimu & Fischer, 2008; North *et al.*, 2011). As such, evidence of G × E by itself does not constitute evidence for local adaptation.

Traditional methods for studying G × E and local adaptation involve reciprocal transplant and common garden experiments (Kawecki & Ebert, 2004; Leimu & Fischer, 2008; Nuismer & Gandon, 2008). However, in sexual organisms, each individual is genetically unique. As such, there is typically no replication at the level of true genotypes, in which case researchers test for an interaction between the population or environment of origin and the population or environment in which fitness is assessed, rather than G × E *per se*. Sexual reproduction also eliminates the possibility of using molecular marker surveys to assess genotype–environment associations in the field, except in rare cases for which one or more genes of known ecological significance have already been characterized (e.g. Schluter *et al.*, 2010). This is true even of studies using experimental inbred lines (e.g. Juenger & Bergelson, 2002) because even a low rate of outcrossing means that inbred lineages in the field will be ephemeral (indeed, experimental lines likely do not exist as identifiable entities in the field), although genetic markers for inbred lines might be used to assess genotype frequencies in artificial populations (e.g. Stratton & Bennington, 1996).

Asexual organisms present an exceptional opportunity to link the ecological characterization of genotypes under experimental conditions to the frequencies of those same genotypes under field conditions (assessed with molecular markers), although this opportunity has seldom been exploited (but see Stratton, 1992, 1994, 1995; Pantel *et al.*, 2011). Previous studies using the apomictic plant species *Erigeron annuus* found strong G × E across experimental disturbance treatments (Stratton, 1992) and across space in unmanipulated fields using sown seeds (Stratton, 1994, 1995). However, these studies did not combine experimental studies of G × E with field surveys of natural populations using molecular markers. Here, we present a study of asexual *Taraxacum officinale* Weber in Wiggers (common dandelion) in which we test for G × E by estimating fitness components in experimentally imposed environmental treatments in a common garden; we then test for local adaptation by assessing the frequency of the same genotypes under different environmental conditions in the field.

Taraxacum officinale, the common dandelion, is a ubiquitous taprooted weed with highly wind-dispersive seeds. It is native to Europe and now found in temperate regions throughout the world, including our study area in Vancouver, British Columbia, Canada (Stewart-Wade *et al.*, 2002). In Europe, *T. officinale* occurs as both sexual diploid and asexual triploid individuals, but in North America, only obligately apomictic asexuals have been found to date (Lyman & Ellstrand, 1984; King, 1993; Stewart-Wade *et al.*, 2002). Previous molecular marker studies in North America have found 1–13 unique genotypes within populations (Solbrig & Simpson, 1974; Lyman & Ellstrand, 1984; Vellend *et al.*, 2009).

Our previous studies on six *T. officinale* genotypes from the Vancouver area have identified two 'winner' and two 'loser' genotypes, showing consistently high or low fitness components, respectively, in a variety of conditions, including a common garden of plants grown individually (Vellend *et al.*, 2009), germination trials in the laboratory (Vellend *et al.*, 2009), as experimental populations in competition with grass (Vellend *et al.*, 2010), and in frequently clipped experimental populations as well as seedling establishment trials in the field (Drummond & Vellend, 2012). A fifth genotype consistently showed intermediate performance, and the sixth was only tested in the common garden experiment where it performed poorly. As such, the maintenance of genetic variation in these populations remains a mystery, as we have found no evidence of trade-offs in terms of fitness components (e.g. germination/establishment vs. competitive ability) or G × E. This contrasts with the work of Solbrig & Simpson (1977), who studied the relative performance of two dandelion genotypes in Michigan and observed a performance trade-off between disturbed and undisturbed habitats.

In residential areas of Vancouver, by far the most common habitat for dandelions is lawns or fields (which may or may not be irrigated in summer), where frequent mowing and trampling reduce overall plant size and seed production. Much larger plants with copious seed production are found in unmown conditions – often of very small area – in and around lawns and fields, such as field edges, fences, benches, rock piles or waste places. Our experiment tested for G × E by measuring the relative performance of six dandelion genotypes in benign (control), clipped (simulated mowing) and drought conditions, whereas the microsatellite survey mapped genotype abundance to mown vs. unmown conditions in the field (summer drought conditions are rare, and it is difficult to confidently assess the drought-proneness of different sites). The latter study allowed us to test for local adaptation and the degree to which this might be countered by the potentially swamping effect of gene flow. We predicted that (i) genotype performance in the control treatment would correspond to the rank order observed in previous experiments, (ii) 'loser' genotypes identified previously would outperform other genotypes in drought and/or mown conditions and (iii) the experimental conditions under which a genotype showed greatest performance would correspond to the conditions in the field where that genotype was most frequently observed.

Materials and methods

Study area

Our study area was the 'west end' of Vancouver, which is a largely residential area (approximately 25 km^2) on Point Grey peninsula, with the University of British Columbia (UBC) campus at its western end (approximately 20% of the study area). This area is ecologically very similar to other (sub)urban areas of the Pacific Northwest of North America. Our experiment was conducted in an open area of Totem Field, a research facility located on the UBC campus. The G × E experiment began with seeds of six dandelion genotypes (comprising the majority of plants in the study area – see Results), which were obtained from a previous common garden experiment. The common garden plants were second-generation offspring of field-collected plants, with the first generation grown in homogenous greenhouse conditions (Vellend *et al.*, 2009). Thus, the seeds used in this experiment were third-generation descendants of field-collected plants, such that any differences between genotypes were heritable genetically, or possibly epigenetically (Verhoeven *et al.*, 2010), across at least three generations.

The microsatellite survey focused on four publicly accessible sites chosen as representative of the study area, in that they presented a mix of typical growing conditions for dandelions in the area (described in the Introduction) and that they were spread apart spatially. There is no unambiguous means of delineating (sub)populations of dandelions in this area, so we were largely interested in the single, aggregate 'population' of dandelions across the four sites, with an assessment of growing conditions for each individual plant (see *Microsatellite analyses*) rather than environmental differences between sites, the latter of which is the more typical focus of local adaptation studies. At all four sites, unmown microsites, which may be only a few meter square or less, occur directly adjacent to, or embedded within, larger mown lawns or fields. Two sites, Totem Field and UBC Farm, were on the UBC campus (1.4 km apart). Totem Field consists of experimental field plots (e.g. common gardens for tree seedlings), a large mowed lawn and a fence around the entire 10-ha property along which many dandelions grow. UBC Farm consists of both crops (e.g. squash, sunflowers, apples) and mowed areas surrounding the crop plots. A third site, Spanish Banks, is approximately 3 km north-east of UBC, where a large nonirrigated lawn is adjacent to a beach along the ocean, with a long strip of boulders (in which dandelions grow) between the lawn and the beach. The fourth site, Chaldecott Park, is a city park of playing fields approximately 4 km east of UBC, where dandelions grow both in the lawn and along fence edges in the baseball field.

G × E Experimental design

Seedlings of the six genotypes were established in potting soil in individual Cone-Tainers (Stuewe and Sons, Tangent, Oregon), distributed among 10 trays (5 reps per genotype per tray, randomly distributed) in a greenhouse environment on 16 June 2010. Two trays were control seedlings, two trays were mown seedlings, and six trays were drought seedlings. Overall, there were 50 replicates of each genotype, with ten replicates of each genotype for control and mow trays and 30 replicates for drought trays. Replication was greatest in our drought treatment because we initially planned to use a binary variable (survival) as the fitness component (ultimately we used time to wilting), which requires greater replication than quantitative measurements (e.g. biomass) to achieve a given level of statistical power. (In the end, replication was sufficient in all three treatments to detect significant differences between genotypes.) Seedlings were thinned to one in each Cone-Tainer and given a small amount of fertilizer solution (N–P–K 15–5–15, approximately 100 ppm) at the start of the experiment. Plants were kept in a greenhouse for approximately 2 weeks until seedlings had been established for approximately 5 days. Seedlings subsequently were transferred to Totem Field. Trays were placed under a wooden frame (60 cm high) covered on top with thick plastic in order to permit control of watering. Plants were initially watered regularly (and equally) for a period of 1 week, and then each tray was randomly assigned one of three treatments. Prior to initiating treatments, individual plants had 4–6 leaves, the longest of which was up to 6 cm long.

'Control' and 'mow' trays were watered every second day, whereas 'drought' trays received no water once treatments started. 'Control' plants were otherwise left unmanipulated, and their total leaf area was estimated three times, at roughly 5 weeks (23 July), 8 weeks (10 August) and 10 weeks (23 August) following the start of the experiment. Total plant leaf area (LA) was estimated from leaf number (N) and the length (L) and width (W) of the longest leaf, using a field-calibrated regression model: $LA = 0.221 \times N \times L \times W$ ($R^2 = 0.95$ based on 56 field-collected plants; Vellend *et al.*, 2009). Control plants were harvested on 30 August 2010, and their roots and shoots were dried and weighed separately. Because plants were clearly pot-bound (i.e. roots growing along pot edges) by the end of the experiment, we analysed leaf area after 5 and 8 weeks as metrics of performance.

To simulate a mowing regime that was at the severe end of what is observed in parks and residential lawns in the area, 'mow' plants had their leaves clipped to 2 cm in length six times over a 7-week period, on 27 July, 30 July, 6 August, 10 August, 1 September and 14 September. Clipping was more frequent during times of more rapid growth (as is typically the case) and prevented leaves from growing longer than approximately 4–5 cm.

We analysed total dry mass, not including clippings, at the end of the experiment as a metric of performance in the mow treatment, as well as dry root mass for illustrative purposes.

'Drought' plants were monitored daily until the death of the last individual on 7 August 2010. Each plant was assigned one of five categories: (i) healthy; (ii) leaves just beginning to wilt; (iii) loss of turgidity in one or more leaves; (iv) loss of turgidity in all leaves, majority of leaves green; and (v) loss turgidity in all leaves, majority of leaves brown. Watering plants following the end of the study period failed to revive any of these plants. 'Drought tolerance' was analysed as both the number of days until stage 4 and stage 5. Biomass was not measured for drought plants because decomposition had proceeded for variable lengths of time across plants by the end of the experiment.

Statistical analyses

Because different performance metrics were, by necessity, measured in different treatments, we first analysed each treatment as a separate experiment using general(ized) linear mixed models, with genotype as a fixed factor and block (i.e. tray) as a random factor. All analyses were conducted in R version 2.10.1 (http://www.r-project.org). For the control and mow treatments, the error distributions of different biomass measurements were modelled as normally distributed (function lme), with significance tests for the fixed effect of genotype calculated based on F statistics (function anova on the lme results). Leaf area measurements in the control treatment were log-transformed to meet the normality assumption. For the number of days until wilting in the drought treatment, we used a Poisson distribution (function lmer) and a likelihood ratio test comparing the full model including genotype and block to a reduced model with only block (function anova comparing two outputs from lmer).

The comparison of results across the three treatments revealed an obvious genotype × treatment interaction. Statistical analysis of this interaction was complicated by the mixture of continuous and count data as response variables in different treatments. We took two approaches, both of which began with calculating relative (rather than absolute) fitness proxies for each response variable (leaf area at 8 weeks for control, total biomass for mow and time to fully wilted for drought) by dividing absolute values by within-treatment means (we were not interested directly in pure treatment effects). First, we conducted a general linear mixed effect model with genotype, treatment (by definition with no effect) and genotype × treatment as fixed effects and block as a random effect. This model assumed normal error distributions, which we recognize to be inappropriate for the count data in the drought treatment (although the approximation is not unreasonable). Second, we conducted a permutation test in which the genotype identities of plants within treatments were shuffled at random 1000 times, after which the F value for the genotype × treatment effect in a regular ANOVA was calculated. For the latter, a P-value was calculated by comparing the F value for the real data to this null distribution.

Microsatellite analyses

To assess the frequencies of our focal genotypes in the field and to test for possible genotype–environment associations, we sampled one fruiting head from each of 26–30 individuals in each of the four sites (described above) on 26–27 August 2010. Although dandelions grow in a variety of conditions in Vancouver, the most visually obvious distinction is between regularly mowed and/or trampled lawns and fields (where plants are typically quite small) and areas where mowing or trampling are rare or absent (at least within a growing season), such as along fence edges, in boulder piles or in agricultural field plots. This binary distinction of microhabitats, which we refer to as 'mown' and 'unmown' for short, was used to assess genotype–environment associations. Although at particular times different areas can appear more or less water stressed, such variability is not visually obvious and we did not conduct detailed analyses of soil moisture availability. As such, we unfortunately could not directly compare the experimental drought results to field patterns, although we chose to retain the drought component of the experiment in this paper as it yielded important insights for at least one genotype.

To generate tissue for DNA analysis, 5–10 seeds per seed head of field-collected plants (described above) as well as plants of our focal genotypes from a common garden (Vellend *et al.*, 2009) were planted in individual pots in a greenhouse during September 2010, and seedlings were thinned to one. After approximately 6 weeks of growth, young, fresh leaves were harvested and approximately 30 mg of tissue (avoiding the mid-rib) was immersed in liquid nitrogen and ground twice for 1 min at 30 Hz using a mixer mill (MM 300, Retsch). DNA was extracted following the protocol reported for chloroplast DNA extraction in Dempewolf *et al.* (2010).

We derived new microsatellite-containing loci from the library of expressed sequence tags for *Taraxacum officinale*, available through the Compositae Genome Project database (http://compgenomics.ucdavis.edu/). Reverse primers were PIG-tailed (Brownstein *et al.*, 1996) with 5′-GTTT-3′ at their 5′ end. Forward primers also had an M13 tail (5′-CAC GAC GTT GTA AAA CGA C-3′) at their 5′ end (Schuelke, 2000). M13 adaptors, homologous to the forward primer tails and labelled with fluorescent dyes, were used for indirect labelling. Polymerase chain reactions (PCR) were performed in a volume of 20 μL containing 10 ng DNA, 0.06 μM of

forward primer, 0.50 μM of reverse primer, 0.50 μM fluorescent dye (6FAM, VIC, NET, or PET), 20 mM Tris–HCl pH 8.8, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.1% Triton X-100, 1 μg of molecular-grade bovine serum albumin, 200 μM of each dNTP and 1 unit *Taq* DNA polymerase. A touch-down PCR protocol (Don *et al.*, 1991) was used to reduce nonspecific fragment amplification, with the following conditions: Initial denaturing step of 3.5 min at 95 °C; 16 touch-down cycles at 94 °C for 30 s; 64–48 °C for 30 s (the annealing temperature started at 64 °C and it was dropped by 1 °C each touch-down cycle); 72 °C for 30 s; 29 cycles at 94 °C for 30 s; 48 °C for 30 s; 72 °C for 30 s; final extension at 72°C for 20 min. A total of 26 primer pairs were used for preliminary tests. After PCR fragments were inspected on 1% agarose gels, 14 primers were retained based on successful amplification and fragment sizes that were not excessively large. Fragment sizes were analysed with an ABI 3730 capillary sequencer (Applied Biosystem, Carlsbad, CA, USA), and ultimately we retained five loci that showed consistent fragment profiles, polymorphism and distinctions among our focal genotypes (see Table 1). Different fluorescent dyes allowed us to subsequently pool the five markers, for which size of amplified fragments were scored in Genemapper version 3.7 (Applied Biosystems) and double-checked visually.

After attrition due to amplification and gel interpretation difficulties, we generated sufficient molecular data for 64 field-collected plants to assign them to one of 11 multilocus genotypes. There was no bias between mown vs. unmown microsites with respect to successful genotyping: 69/112 sampled plants (62%) were found in mown microsites compared to 38/64 (59%) genotyped plants. As a first cut, we assigned plants to multilocus genotypes using the program GENODIVE (Meirmans & Van Tienderen, 2004), with a threshold difference of zero (i.e. only plants with precisely identical microsatellite alleles at all loci were grouped into the same genotype). Because minor errors in PCR or peak scoring, as well as mutation, can prevent true clone mates from being assigned to the same genotype, we subsequently went through the results by hand to allow plants to belong to the same genotype if they differed only by the presence or absence of a single allele at one locus or if the size of one allele differed by 1–2 base pairs.

Results

Significant performance differences between genotypes were observed in all three treatments. In the control treatment, differences between genotypes for total leaf area at 5 weeks ($F_{5,53} = 8.71$, $P < 0.001$) and 8 weeks ($F_{5,53} = 2.60$, $P = 0.035$) were similar to differences seen in earlier experiments (Fig. 1a). Note that in Figs 1 and 2, genotypes are ordered according to their average rank performance under benign conditions, which is very strongly supported by several previous experiments (Vellend *et al.*, 2009, 2010; Drummond & Vellend, 2012) as well as this study (e.g. Fig. 1a). In the mow treatment, differences between genotypes in total dry biomass ($F_{5,53} = 9.82$, $P < 0.001$) was almost entirely due to root biomass ($F_{5,53} = 9.03$, $P < 0.001$), and in contrast to the control treatment (and all previous experiments), genotypes 24 and 53 performed best (Fig. 1b). The most drought-resistant genotype was 64 (Fig. 1c), which was also a poor performer in the control treatment and previous experiments. Strong differences were observed between genotypes for time to full wilting (Δ – log likelihood$_5 = 14.33$, $P < 0.001$) and partial wilting (Δ – log likelihood$_5 = 17.09$, $P < 0.001$). Performance differences varied strongly among treatments (Fig. 1), with significant results in a combined mixed model on relative fitness values ($F_{10,275}$ (genotype × treatment) = 10.36, $P < 0.0001$) and via permutation test ($P < 0.001$).

Five of the six focal genotypes were found in the four sites sampled for microsatellite analysis, and these genotypes made up > 80% (55/64 plants) of plants in these sites (Table 2). We detected six additional rare genotypes (Table 2). Among the 11 multilocus genotypes, identical alleles were never shared at more than one locus, and

Table 1 Characteristics of microsatellite markers used in this study. Locus names come from the Compositae Genome Project database (http://compgenomics.ucdavis.edu/). Note: the range size is not necessarily a multiple of the repeat motif size because mutations in the flanking regions might have occurred.

Locus name	Primer direction	Primer sequence (5′–3′)	Motif	Allele size range (bp)	No. of alleles
CL4019Contig1	M13-Forward	CACGACGTTGTAAAACGACAAGACGGTGGAACTGAATGC	TC	342–378	12
	PIG_tail-Reverse	GTTTGGATCGAATACATCCCCAAA			
CTOY10260	M13-Forward	CACGACGTTGTAAAACGACCAGCAGAGATTGGGTGTTGA	GAA	272–323	10
	PIG_tail-Reverse	GTTTCGATCTTGCATCTCCTCA			
CTOZ1579	M13-Forward	CACGACGTTGTAAAACGACACAGATGAAAGGCAAGCAGA	TC	280–298	12
	PIG_tail-Reverse	GTTTCTATCCACCACACAAAAGG			
CL1729Contig1	M13-Forward	CACGACGTTGTAAAACGACCCATGGATGAGCACAAAGTG	CT	126–169	11
	PIG_tail-Reverse	GTTTCCCACACATAGAGCAAAACA			
CL5650Contig1	M13-Forward	CACGACGTTGTAAAACGACTCACACAGTCGCTCAAATCG	TC	223–237	2
	PIG_tail-Reverse	GTTTAATGGTGGATCGGGGTAAAT			

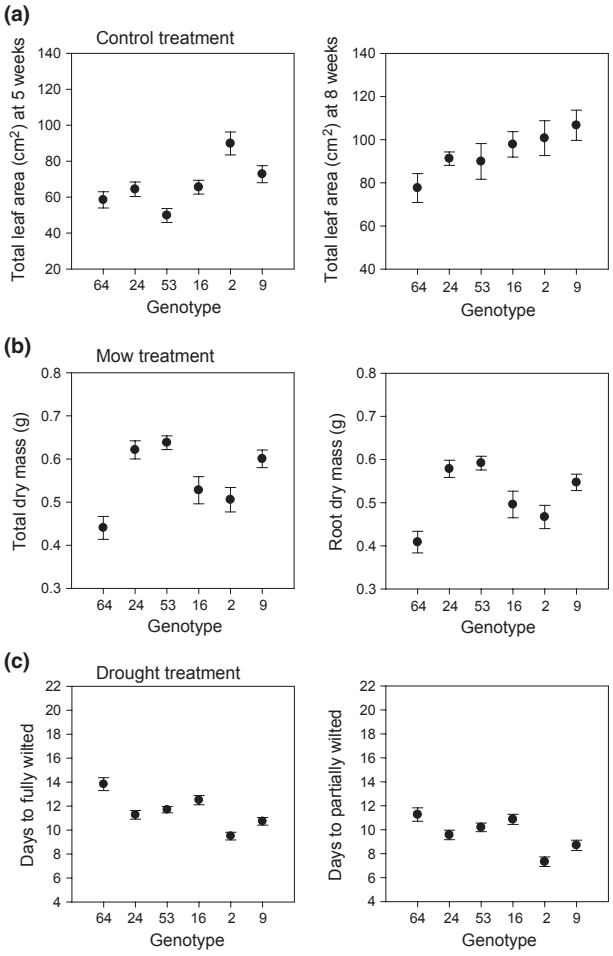

Fig. 1 Performance measures of six dandelion genotypes in three treatments. The order of genotypes on the *x*-axis corresponds to average performance under benign growing conditions in previous experiments (Vellend *et al.*, 2009, 2010).

this was the case for only 5 of 110 pairwise comparisons, indicating excellent capacity of these markers to discriminate asexual lineages. With data pooled across sites, there were strong and significant differences between genotypes for their frequency in mown vs. unmown microhabitats (Fisher's exact test, $P < 0.001$; Table 2). Genotype 24 occurred significantly more often (22/23 occurrences) in mown microsites than expected given the frequency with which they were sampled (59% of plants were in mown microsites; binomial probability = 0.0008 for ≥ 22/23 occurrences). Genotypes 2 and 9 (pooled due to low sample size) occurred less often in mown microsites than expected at random (binomial $P = 0.0057$ for ≤ 3/16 occurrences). Although we recognize that the small number of focal genotypes allows only very weak tests for correlations between performance and frequency or microhabitat associations, nonetheless, there appeared to be no relationship between performance rank order under benign conditions (from previous experiments and the control treatment here) and overall frequency (Pearson's correlation, $r_4 = 0.02$, $P = 0.97$, Fig. 2a), but a tendency for a positive correlation between benign performance rank and the proportion of plants was found in unmown microsites ($r_3 = 0.81$, $P = 0.09$, Fig. 2b).

Discussion

We found evidence of strong G × E interactions under experimental mown, drought and control environments, and by studying an asexual species, *T. officinale*, we were able to directly link these results to field-level genotype frequencies. Collectively, our results are strongly indicative of local adaptation in these sites, within which our six focal genotypes represent over 80% of individuals.

For four of our genotypes (2, 9, 16, 24), the experimental and field survey results, combined with previous studies, allow clear interpretations with respect to local adaptation and plant strategies (sensu Grime, 2001). Genotypes 2 and 9 were more common in unmown than in mown locations (Fig. 2b, Table 2), and their relative fitness was highest under relatively benign conditions in this study (Fig. 1a) and even more evidently in several previous, independent experiments (Vellend *et al.*, 2009, 2010; Drummond & Vellend, 2012). This strongly

Table 2 Frequencies of dandelion genotypes in mown and unmown microhabitats in four populations in Vancouver, British Columbia.

		Focal genotypes					Other genotypes					
Population	Microhabitat	24	53	16	2	9	A	B	C	D	E	F
Totem Field	Mown	11	–	1	–	–	–	–	–	–	–	–
	Unmown	1	–	–	1	–	–	–	–	1	–	–
UBC Farm	Mown	–	3	2	–	–	–	–	–	–	–	–
	Unmown	–	2	2	2	–	–	–	–	–	1	3
Spanish Banks	Mown	3	–	3	–	3	–	–	–	–	–	–
	Unmown	–	–	–	2	6	–	–	1	–	–	–
Chaldecott Park	Mown	8	–	1	–	–	1	2	–	–	–	–
	Unmown	–	–	2	2	–	–	–	–	–	–	–
Total	Mown	22	3	7	0	3	1	2	0	0	0	0
	Unmown	1	2	4	7	6	0	0	1	1	1	3

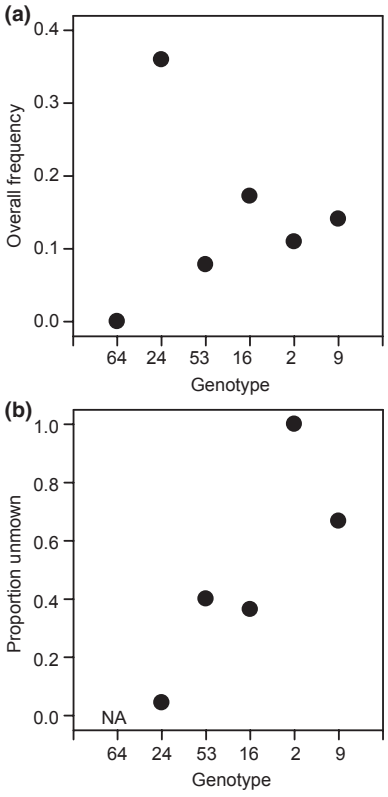

Fig. 2 Frequencies of six dandelion genotypes across four populations and the proportion of individuals of each genotype found in 'unmown' microhabitats.

suggests that, for these genotypes, the capacity for rapid growth, competitive ability and seed production trades off with low fitness under stressful or disturbed conditions. Genotype 16 appears to have a generalist strategy, with intermediate relative performance under various experimental conditions (Fig. 1) and a relatively even spread across mown and unmown conditions in the field (Fig. 2b). Genotype 24 was found almost exclusively under mown conditions (Fig. 2b), where its relative fitness is highest (Fig. 1b), strongly suggesting that disturbance tolerance trades off with competitive ability and rapid growth under benign conditions. Genotype 24 was also the most abundant in the field (Fig. 2a), likely because mowed lawns are the predominant habitat type for dandelions in Vancouver (and other urban areas).

The dominance of genotype 24 in mown habitats in the field suggests that, despite what is likely a considerable input of seeds of genotypes 9 and 2 into lawns, and therefore potentially high gene flow, selection is strong enough to largely restrict them to unmown microsites. We expect high seed movement from unmown to mown conditions for several reasons: (i) lawns are the most common habitat for dandelions in Vancouver, with unmown microsites often embedded within or adjacent to large areas of mowed lawn; (ii) even in a common environment, individuals of genotypes 2 and 9 produce far more seeds than genotype 24 (Vellend *et al.*, 2009), and this difference is almost certainly greatly magnified by the environmental difference between mown and unmown microsites; (iii) dandelion seeds are highly dispersive (on a windy day one can readily observe dandelion seeds blowing from large unmown plants onto mown lawns). Likewise, selection appears to severely depress genotype 24's frequency under benign conditions. Although individual plants of genotype 24 produce relatively few seeds, the abundance of this genotype plus highly dispersive seeds indicates that gene flow from unmown to mown habitats is also likely high.

Interpretations regarding the plant strategies of genotypes 64 and 53 are necessarily more speculative, because we have no direct information on the frequency of 64 under different conditions in the field, and genotype 53 was included in only one of our previous studies (a common garden experiment). However, genotype 64, which was clearly most tolerant of drought conditions (Fig. 1c), has distinctly reddish achenes, which are quite common on eastern Vancouver Island (MV, personal observation) where summer rainfall is very low relative to the broader region. These results and observations are at least consistent with local adaptation of genotype 64 to water-stressed conditions at the expense of performance under more mesic growing conditions. From genotype 53, G × E results were quite similar to those for genotype 24 (Fig. 1), although it was found at much lower frequency in the field and little can be said about microhabitat affinities. We can also only speculate as to why a previous experiment, in which small populations of each genotype and combinations of genotypes were grown in clipped and unclipped treatments in the field (Drummond & Vellend, 2012), failed to reveal any advantage of genotype 24 in the clipped treatment. In this previous experiment, to minimize seedling or plant death, we clipped them only at monthly intervals, with clippers rather than a mower, and only to sod level (approximately 5 cm height), which (in retrospect) seems unlikely to have represented a sufficiently strong selective pressure to shift the balance in favour of genotypes adapted to mown conditions.

We observed G × E interactions at very small spatial scales in this study, with small unmown microsites (e.g. a single park bench or approximately 20-m stretch of fence in a baseball diamond) largely interspersed within mown areas of several hectares. As such, a typical population or site-level reciprocal transplant experiment would have likely either failed to detect local adaptation or, at best, detected only a weak signal. By studying an asexual species and using microsatellite markers to tie individuals of known genotype to precise locations in natural field populations, we were able to detect clear local adaptation created by small-scale G × E interactions. Experiments with the apomict *Erig-*

eron annuus detected G × E interactions at scales as small as 10–20 cm using experimentally sown seeds (Stratton, 1994, 1995), although it was not determined whether this translated into differential distributions of genotypes across environmental conditions in natural populations. In studies of *Arabidopsis thaliana*, G × E was found at distances of 10–50 cm (Stratton & Bennington, 1996), although these were entirely artificial populations. In natural populations of sexual species, local adaptation has also been shown to occur over very small spatial scales in systems with strong underlying environmental or phenological gradients (Linhart & Grant, 1996), such as mine tailings adjacent to unpolluted soils (e.g. Antonovics & Bradshaw, 1970; Antonovics, 2006; Levin, 2009). However, most studies of local adaptation consider environmental variation at the among-population level (Kawecki & Ebert, 2004; Leimu & Fischer, 2008). At very small scales, gene flow is assumed to be quite high and therefore a potent force opposing the evolution of local adaptation (Lenormand, 2002; North *et al.*, 2011).

In addition to the long-standing interest in the *maintenance* of genetic diversity, ecologists are also becoming increasingly interested in the *consequences* of genetic diversity (Hughes *et al.*, 2008). Genetic diversity within a population is a critical component of an organism's ability to adapt to new environments (e.g. Dlugosch & Parker, 2008), but initial or standing genetic variation can also have important short-term consequences (Hughes *et al.*, 2008), especially for colonizing exotic species, such as dandelions, for which there is huge variation in the identity and variety of genetic variants present upon initial colonization (e.g. Lavergne & Molofsky, 2007; Vellend *et al.*, 2007). Our results suggest that if a site is invaded by only a subset of genotypes, the outcome of the invasion depends strongly on which genotypes comprise the subset. For example, whether or not a mowed lawn habitat receives seeds of genotype 24 may determine both the probability of a successful invasion as well as the eventual abundance if an invasion is successful. A number of studies have found increased biomass production in populations with larger numbers of genotypes, in part because having more genotypes increases the chances of including the 'right' genotype (reviewed in Hughes *et al.*, 2008). Additionally, our results suggest that the positive effect of genetic diversity on population growth and productivity might be even stronger in heterogeneous environments, in which different genotypes maximize productivity in different microsites. This is an easily testable hypothesis for future studies.

The present study had two limitations worth noting. First, without characterization of the moisture status of microsites, we were unable to draw strong conclusions about local adaptation to drought. Second, the sample size of genotyped plants was less than initially hoped, given difficulties with DNA amplification and gel interpretation, such that relatively few individuals were characterized for individual genotypes. However, these limitations did not prevent us from detecting the two overarching patterns of interest: G × E interactions and a clear correspondence between genotype performance in different experimental treatments and microenvironmental affinities in the field.

In sum, we found strong coherence between experimental G × E interactions and field-level genotype frequencies, detected using microsatellites, providing clear evidence of local adaptation. Using an asexual species afforded us true replication at the level of the genotype and allowed us to use molecular markers to directly assess genotype–environment associations within a heterogeneous field situation. The results of our study indicate that differential selection among habitat types is strong enough to drive local adaptation in these dandelion populations and that selection happening at small spatial scales may easily be overlooked by traditional methods used for detecting local adaptation.

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