Report

Current Biology

Whole-Genome Hitchhiking on an Organelle Mutation

Highlights

- Selection on organelles can effect nuclear genetic diversity via genetic hitchhiking
- Organelle-mediated genetic draft is an underappreciated evolutionary phenomena

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In Brief

Flood et al. discover that strong selection on a chloroplast gene has extended to the nuclear genome, which has hitched a ride along with the selected chloroplast. This is the first description of organelle-mediated genetic draft and shows that selection on organelles can directly impact nuclear genetic diversity.

Flood et al., 2016, Current Biology *26*, 1306–1311
May 23, 2016
http://dx.doi.org/10.1016/j.cub.2016.03.027

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Whole-Genome Hitchhiking on an Organelle Mutation

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SUMMARY

Strong selection on a beneficial mutation can cause a selective sweep, which fixes the mutation in the population and reduces the genetic variation in the region flanking the mutation [1–3]. These flanking regions have increased in frequency due to their physical association with the selected loci, a phenomenon called "genetic hitchhiking" [4]. Theoretically, selection could extend the hitchhiking to unlinked parts of the genome, to the point that selection on organelles affects nuclear genome diversity. Such indirect selective sweeps have never been observed in nature. Here we show that strong selection on a chloroplast gene in the wild plant species *Arabidopsis thaliana* has caused widespread and lasting hitchhiking of the whole nuclear genome. The selected allele spread more than 400 km along the British railway network, reshaping the genetic composition of local populations. This demonstrates that selection on organelle genomes can significantly reduce nuclear genetic diversity in natural populations. We expect that organelle-mediated genetic draft is a more common occurrence than previously realized and needs to be considered when studying genome evolution.

RESULTS AND DISCUSSION

In outcrossing species, the extent to which selection on a locus in the nuclear genome can distort neighboring gene frequencies is restricted by recombination [1, 2]. In non-recombining genomes, such as those of mitochondria, chloroplasts, and bacteria, selection at a single locus can, however, result in genome-wide selective sweeps [5]. Although the potential consequences of selection on organellar genomes have been frequently discussed [6], there has been little recognition that the effect of selection on organelles may extend to the nuclear genome as well [7, 8].

Here we make use of the natural distribution of an easily detectable chloroplast mutation to study the impact of selection on organelles in a wild plant population. The chloroplast mutation confers resistance to triazine herbicides, which were widely used along British railways from 1957 until their discontinuation in 1992 due to environmental concerns. Triazines inhibit photosynthesis by competing with plastoquinone for the Q_B binding site of the D1 protein of photosystem II (PSII), thereby inhibiting photosynthetic electron flow [9]. The D1 protein is common to all photosynthetic eukaryotes and is encoded by the chloroplastidic *psbA* gene. A conserved Ser-264-Gly amino acid substitution confers resistance via the removal of a hydrogen bonding site, which prevents triazines from binding to D1 [9]. This particular amino acid substitution has evolved at least 73 times across multiple plant species [10]. In addition to conferring resistance to triazine herbicides, this substitution also reduces the affinity of the Q_B site for plastoquinone, thereby reducing the efficiency of PSII [9] (Figure 1). This reduction in PSII efficiency limits carbon assimilation under most environmental conditions [11] and consequently results in a fitness cost [12, 13]. Resistant genotypes are therefore expected to be strongly selected for by triazine application but gradually removed from the population in its absence [12, 13].

Triazine resistance in the wild plant species *Arabidopsis thaliana* was first detected in 1988 at Ely railway station, Cambridgeshire, UK, after which the resistant genotype is named [14]. From an existing dataset of 149 nuclear SNPs [15], we identified four genotypes from Liskeard, Cornwall, over 400 km from Ely (Figure 2; Table S1), that were identical to the Ely genotype for all SNPs. Progeny of all four genotypes tested positive for the *psbA* mutation and exhibited atrazine resistance (Table S2), suggesting that resistance may be associated with a single nuclear haplotype. To estimate the frequency of triazine-resistant genotypes and detect the occurrence of organelle-mediated nuclear genetic hitchhiking, we sampled a total of 60 populations of *A. thaliana* at 47 distinct locations along British railways in the east and southwest of England and tested for the presence of triazine resistance and the nuclear genetic diversity of resistant and non-resistant plants. A subset of 36 populations was specifically sampled alongside railway tracks, where selection for chloroplast-mediated triazine resistance was known to occur, whereas the remaining populations were sampled at least 500 m from the nearest railway track, where no record of triazine application was noted and thus strong selection on the organelle mutation was unlikely.

Of the 573 plants assayed, 51 carried the *psbA* mutation (Figure 2; Table S1). These resistant plants were found at 12 of the 47 sampled locations and were all located on or next to railway

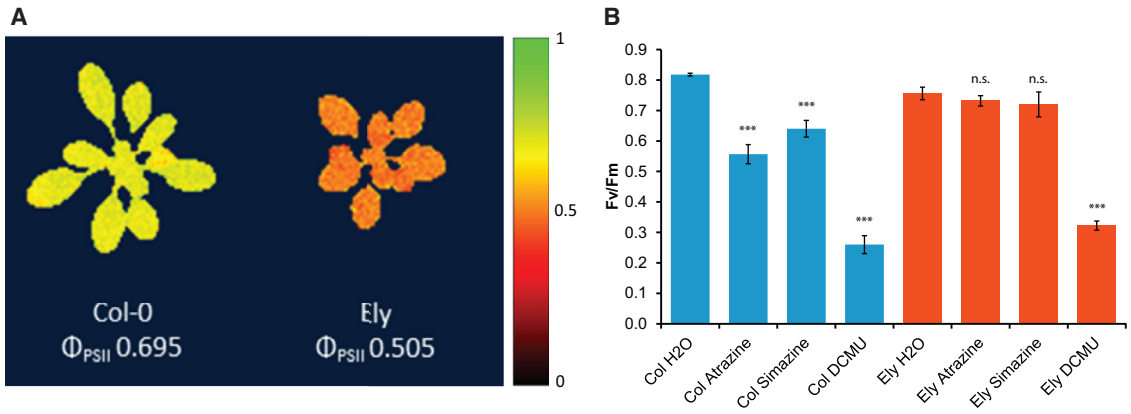

Figure 1. Photosynthetic Performance of Triazine-Resistant and -Susceptible Plants

(A) False-color chlorophyll fluorescence image of light-adapted photosystem II operating efficiency (Φ_{PSII}).

(B) Dark-adapted chlorophyll fluorescence (F_v/F_m) of susceptible (Col-0) and resistant (Ely) genotypes in response to incubation in water (H_2O), atrazine, simazine, and DCMU. ***$p < 0.0001$; n.s., not significant.

See also Table S2.

tracks. The mean frequency of resistance near railways was 0.12. None of the 24 non-railway populations yielded any resistant plants, confirming that the resistance is positively associated with railways ($p < 0.0001$ for a test of allele frequency association, and $p < 0.001$ with a Fisher exact test based on the presence/absence in railway/non-railway populations) and is thus likely to be under anthropogenic selection. Genotyping of the 51 resistant and 342 non-resistant plants for 30 polymorphic, nuclear SNPs (Figure S1; Table S1) showed that all resistant plants belonged to one multi-locus haplotype that is absent from non-resistant genotypes (Figure 3). The expected population frequency of such a haplotype by random association of SNPs is below 0.0001, strongly suggesting that these 51 identical haplotypes originated from a single ancestor. This ancestor probably does not originate from around Ely, although due to the lack of a clear pattern of isolation by distance in British *A. thaliana* [15] it was impossible to pinpoint a likely site of origin (Figure S2). Although the resistant haplotype was not the only nuclear haplotype found at multiple locations, it was the most common and the only one to be positively associated with railways.

Based on these results, we conclude that triazine resistance arose once among the tested populations and then spread in a single genetic background over a large area (Figure 2). The single origin of all resistant genotypes is striking, as the entire nuclear genome appears to have hitchhiked along with the chloroplast mutation [4]. This nuclear genetic hitchhiking was most likely facilitated by the strength of selection, the reduction in effective recombination due to the high level of inbreeding in *A. thaliana*, and the haploid nature of the chloroplast genome, which renders all mutations dominant. The association of the triazine-resistant genotype with railways likely facilitated its dispersal [16], but cannot explain its abundance alone. If the abundance of the resistant genotype was purely a product of dispersal by railways, then other genotypes would also be expected to show similar dispersal patterns. However, none of the other haplotypes found at multiple locations were associated with railways, and thus the abundance of the resistant haplotype is not just a product of its association with railways but required the selective advantage conferred by triazine resistance to reach its current frequency. The current British rail network comprises 32,000 km of track and 30,000 ha of lineside vegetation [17]. During the period of triazine application (at most 35 years), the habitat available to resistant *A. thaliana* was large and probably free from competition, as no other triazine-resistant species has been reported on British railways [10].

This study provides a particularly favorable context in which to detect an organelle-mediated selective sweep, as the occurrence of natural selection on a single organellar locus can be demonstrated by virtue of prior knowledge of the mutation, localized herbicide application, and the possibility of sampling systematically at sites where the selection pressure was known to occur. We thereby overcome the usual limitations on inferring selection from population-genetic data, in which observed patterns of diversity can often not be disentangled from those caused by demographic history. Nevertheless, none of the three conditions leading to nuclear genome hitchhiking—strong selection, inbreeding, and haploid organelles—are unique to the case we describe. Inbreeding is common in many organisms, organelles are always haploid, and strong selection is common and expected to become more so in heavily human-influenced environments [3, 18]. Thus, we expect that organelle-mediated selective sweeps resulting in nuclear genome hitchhiking are more common, although difficult to detect empirically. It is worth noting that such genome-wide sweeps can also occur due to selection at the autosomal level; however, we have focused here on the potential for organelles to mediate trans-genome hitchhiking, as the effect of selective sweeps of autosomal origin has been studied previously [3].

The population of photosynthetically impaired plants persisted for 22 years after the cessation of triazine use. In crops, the *Ser-264-Gly* mutation reduces seed yield by 22%–36% [11]. Although many crops exhibit photosynthetically limited growth under agricultural conditions [11, 19], this is not thought to be the case in natural ecosystems, where resource restriction and competition are more likely to be limiting [20]. The picture that emerges for natural populations is that the fitness cost of triazine

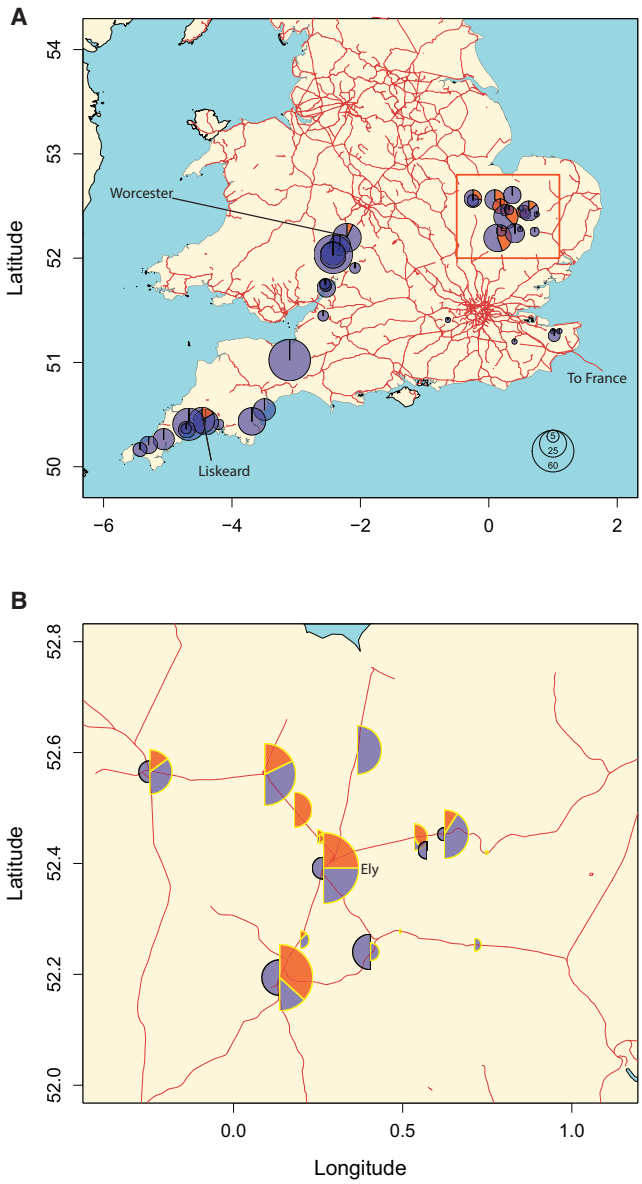

Figure 2. Map Showing the Distribution of Sampling Sites and Number of Plants Sampled

(A) Map of all populations sampled; locations where triazine-resistant plants were found outside the red-boxed area are labeled. Circle size indicates the number of plants. Red fractions indicate atrazine-resistant plants; blue fractions indicate atrazine-susceptible plants.

(B) Map of the area surrounding the site of the original Ely accession (red box in A). Left semicircles with black circumference represent accessions from non-railway sites, and right semicircles with yellow circumference represent accessions from railway sites. Semicircle area is proportional to sample size; blue represents susceptible plants and red represents resistant plants; railways are indicated with brown lines.

See also Figure S2 and Table S1.

resistance in the absence of triazines is environment specific, being less at low temperatures and low light levels [21] and greater in the presence of biotic stresses [22, 23]. A simple population-genetic model shows that, given estimates of migration of 1%–2% per year [24] and a selective cost, s, of 0.22–0.36, it would take an estimated 10–19 generations to retain frequencies equal to or higher than those observed for railway populations. Due to seed bank persistence and recruitment, *A. thaliana* has an average generation time of 3–4 years [25]. This would equate to 30–76 years to reach observed frequencies, which is compatible with the time since discontinuation of triazines. In addition, the 35-year period of triazine use allowed the build-up of monogenic stands of resistant individuals, with a large seed bank to provide resistant populations with an additional buffer against extinction [24]. Thus, the most likely scenario is that the dispersal of the resistant genotype was aided by trains and that it rose to a very high frequency in some areas due to strong selection by triazine application. The absence of the resistant genotype in some areas may be due to limited dispersal or subsequent extinction, although in several locations very few plants were found, so it may be present and just not detected.

The evolution and spread of this triazine-resistant genotype along British railways are a conspicuous example of anthropogenic disturbance greatly altering the population structure of a wild plant species [26]. This organelle-mediated sweep is a striking example of linked selection, reaffirming the significant role that selection can play in genome evolution [27], and how selection on organelles can have a large and, thus far, underappreciated role in nuclear genome diversity and evolution.

EXPERIMENTAL PROCEDURES

Plant Collection and Cultivation

Seed was collected in August 2012 around Ely railway station and again at Ely and additional sites in Cambridgeshire and Suffolk during May and June 2013. A final round of sampling was conducted in May 2014 in the southwest of England, from Cornwall to Herefordshire. When collecting, both leaf and seed material were taken on or near railway-associated land and, where possible, additional samples were taken some distance away (>500 m). This was done in order to sample a putatively local population not associated with the railway. GPS data were recorded for all plants sampled. Sampled leaf material was stored with silica gel to ensure rapid desiccation and optimal preservation of the DNA. Upon return to the laboratory, seed was sown on wet filter paper and stratified at 4°C for 4 days to break dormancy. The seed was then sown in a greenhouse on rockwool blocks (www.grodan.com). Depending on seed availability, up to four seeds were sown per block with four blocks per genotype. Extra seedlings were removed after 2 weeks, leaving one plant per block, and one leaf was taken from each accession for genotyping. Genotypes that showed no signs of flowering after 5 weeks were moved to a cold room for 8 weeks of vernalization. Seed was harvested from all lines and stored (see Table S1 for a full list).

Genotyping

A 350-bp region around the chloroplast *psbA* mutation was amplified using PCR and primer sequences 5′-CTATGCATGGTTCCTTGGTAACTTC-3′ and 5′-CGTTCATGCATAACTTCCATACCA-3′. A primer annealing temperature of 54°C was used during PCR. The PCR product was then digested overnight at 37°C using the restriction enzyme FspBI, which only cleaves wild-type sequences, resulting in 210- and 140-bp fragments. Genotypes with the mutated sequence were not cleaved, allowing easy identification of resistant genotypes. Three hundred and ninety-four lines were further genotyped for 39 nuclear SNPs described previously [28]. Nine SNPs were excluded from further analysis, as they were either monomorphic or had more than 10% missing data. The remaining 30 SNPs were used in all further analysis (Figure S1; Table S1).

Phenotyping

The phenotyping procedure exploits the mechanism of triazine toxicity, its effect on quantum efficiency of PSII electron transport, and the use of chlorophyll fluorescence to measure PSII quantum efficiency. Normal PSII electron

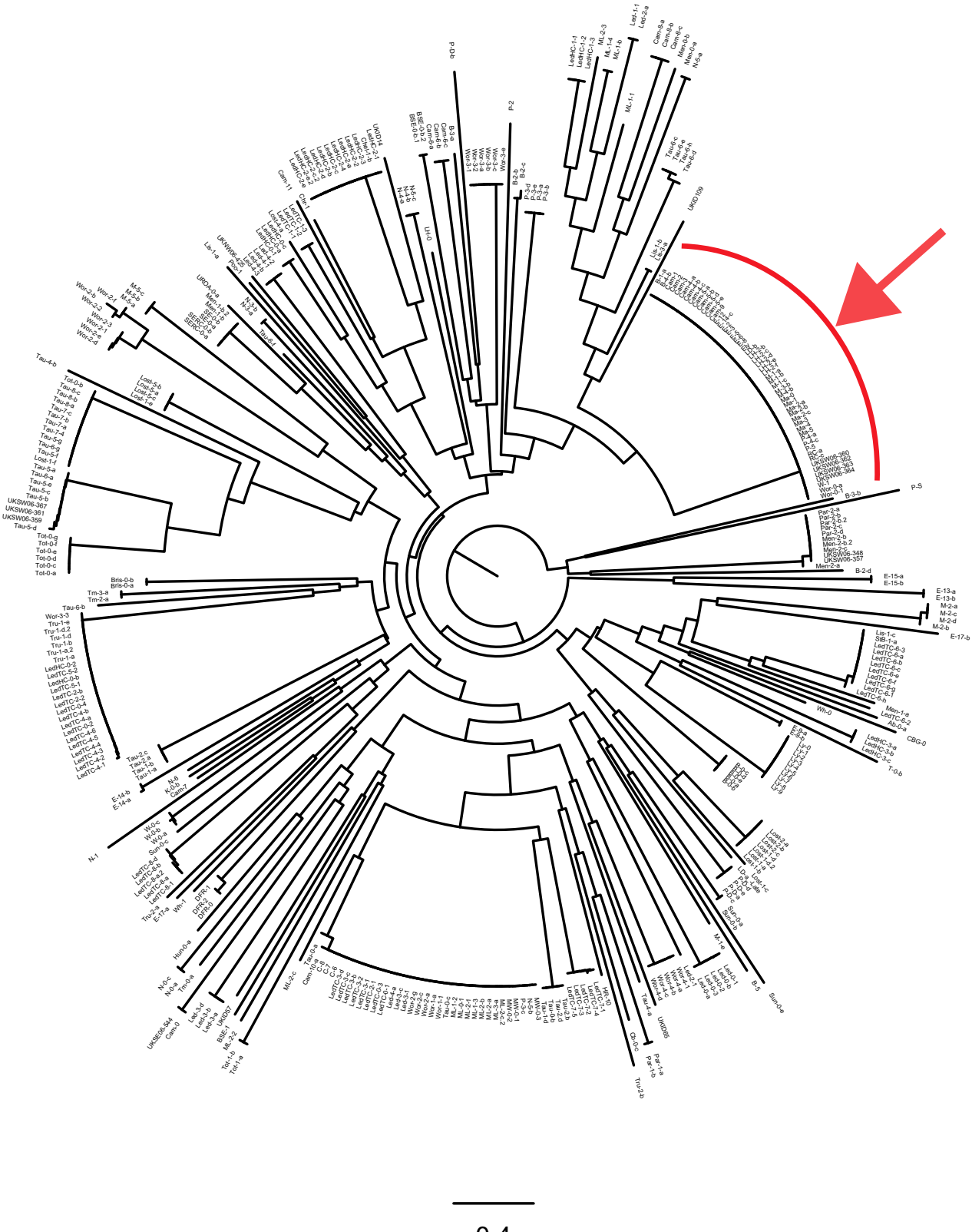

Figure 3. Neighbor-Joining Tree of 573 Sampled *A. thaliana* Plants Genotyped with 30 SNPs

The cluster of 51 genetically identical plants, indicated with the red arrow, contains all, and only, the triazine-resistant plants. See also Figure S1 and Table S1.

transport depends on the photochemical reduction of Q_A (the first stable electron acceptor of PSII) and the forward passage of this electron via Q_B (the electron acceptor for Q_A^-) to the rest of the photosynthetic electron transport chain and photosystem I. The efficiency of this process can be conveniently measured using chlorophyll fluorescence, in particular by means of two relative chlorophyll fluorescence yields, F_o and F_m [29]. These yields correspond, respectively, to those of open PSII (i.e., when forward electron transport occurs with maximum efficiency) and closed PSII (i.e., when forward electron transport occurs with zero efficiency, owing to the reduction of Q_A). The quantum yield of PSII electron transport can be calculated as $(F_m - F_o)/F_o$, which is often abbreviated as F_v/F_m, where $F_v = F_m - F_o$. In wild-type plants the presence of triazine prevents the binding of plastoquinone to form Q_B, thus preventing forward electron transport, resulting in the accumulation of Q_A^- and a PSII quantum yield of zero. An accumulation of Q_A^- will result in the increase in the level of chlorophyll fluorescence [29]. In the triazine-resistant genotype, plastoquinone continues to bind in the presence of triazines, forming Q_B, but there is a reduction in PSII quantum yield in the presence or absence [14] of atrazine. One resistant and one susceptible genotype (based on the PCR assay previously described) from each location sampled were sown on rockwool blocks. Three weeks after sowing, leaves were cut and placed in a Petri dish containing wet filter paper soaked in either water or a 100 μM solution of atrazine. The leaves were vacuum infiltrated as described in [14] and dark-adapted for 30 min, upon which the chlorophyll fluorescence parameters F_o and F_m were measured, and the F_v/F_m value was calculated [29] using a FluorCam 800MF chlorophyll fluorescence imager (www.psi.cz). In susceptible genotypes treated with atrazine, the fluorescence measuring beam closes some PSII reaction centers by reducing Q_A to Q_A^-, resulting in a higher F_o (strictly no longer an F_o) and a decreased F_v/F_m. Resistant plants show no decline, allowing easy assessment of atrazine tolerance. Cross-tolerance to simazine was confirmed using Ely and Col-0 only (Figure 1). These genotypes were also assayed for resistance to an alternative PSII inhibitor, DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), for which the Ser-264-Gly amino acid substitution in D1 should provide no cross-tolerance (Figure 1; Table S2).

Relatedness and Geographic Assignment

A simple dissimilarity matrix was calculated by taking the Euclidean distance based on 30 SNPs and used to construct a neighbor-joining tree (Figure 3). Continuous geographic patterns of multi-locus variation were modeled by spatial interpolation using 4,377 regularly spaced grid points selected within a polygon encompassing the United Kingdom and a part of continental Europe. First, the 149-SNP marker matrix for 2,567 geo-referenced individuals was reduced to 40 independent axes of variation (principal components; PCs) by principal component analysis. After exclusion of the Ely accession (also known under accession codes PHW31, CS28631, and 7502) from the matrix, PCs were averaged over 483 unique locations and a geo-spatial model was fitted to each PC using the function Likfit in the R package geoR (http://www.leg.ufpr.br/geoR/). The predicted value for individual PCs at each grid point was then generated by model-based kriging, using the function krige.conv and using the fitted spatial models as an input. Finally, the Euclidean distance along the 40 PCs between each grid point and Ely genotype was calculated for every grid point, resulting in a map of estimated genetic distance to the Ely genotype (Figure S2).

Testing for Association with Railways

Haplotype frequencies were calculated for the 393 individuals genotyped for 30 polymorphic nuclear SNPs, excluding the Ely reference genotype. The expected haplotype frequency under the assumption of independence between loci was calculated for each haplotype as the product of allele frequencies. At each location, haplotype frequencies were calculated for railway populations and non-railway controls. We tested for positive association with railways for the Ely haplotype and the 12 haplotypes occurring in more than one location, using a generalized linear model with a binomial link function. A more conservative Fisher exact test for differences in the presence of resistant phenotypes between railway and non-railway populations was also performed to correct for dependence due to inbreeding.

Numerical Simulation of Persistence

Persistence of the mutation was simulated with a deterministic model assuming migration from an infinite wild-type population into a mutant population and subsequent selection against the mutant allele. Starting with a wild-type frequency, p, of zero, and assuming $p = 1$ for the infinite wild-type population, each generation would see a change in wild-type frequency according to $p' = p(1 - m) + m$ and $p'' = p' / (w(1 - p') + p')$, with m and $w = 1 - s$ being the proportion of immigrants and the relative fitness, respectively.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.03.027.

AUTHOR CONTRIBUTIONS

P.J.F., J.H., and M.G.M.A. conducted fieldwork. P.J.F. and F.B. propagated, genotyped, and screened material. C.B.d.S. conducted SNP genotyping. P.J.F. and J.v.H. analyzed data. P.J.F., J.H., M.G.M.A., and J.v.H. designed the study and wrote the paper with comments from all other authors.

ACKNOWLEDGMENTS

We thank P. Williams for details on the original collection site for Ely, and E. Holub for details on lines collected from Liskeard. We thank J. Hoedemaker, S. Schoustra, and N. Aarts for help with seed collection, J. Snel for advice on conducting the atrazine treatments, A. Wright and D. Hayward for information regarding current and past herbicide use on British railways, T. Morton, J. Bergelson, and M. Horton for seed of Ely relatives and details regarding their origin, and T. Bisseling, J. Kirwan, M. Koornneef, S. Schoustra, E. Wijnker, and B. Zwaan for comments on the manuscript. This work was financially supported by an ALW/TTI-Green Genetics grant from the Dutch Organization of Scientific Research (NWO).

Received: October 15, 2015
Revised: January 22, 2016
Accepted: March 10, 2016
Published: April 28, 2016

REFERENCES

1. Cutter, A.D., and Payseur, B.A. (2013). Genomic signatures of selection at linked sites: unifying the disparity among species. Nat. Rev. Genet. *14*, 262–274.
2. Neher, R.A. (2013). Genetic draft, selective interference, and population genetics of rapid adaptation. Annu. Rev. Ecol. Evol. Syst. *44*, 195–215.
3. Andersen, E.C., Gerke, J.P., Shapiro, J.A., Crissman, J.R., Ghosh, R., Bloom, J.S., Félix, M.-A., and Kruglyak, L. (2012). Chromosome-scale selective sweeps shape *Caenorhabditis elegans* genomic diversity. Nat. Genet. *44*, 285–290.
4. Maynard Smith, J., and Haigh, J. (1974). The hitch-hiking effect of a favourable gene. Genet. Res. *23*, 23–35.
5. Lang, G.I., Rice, D.P., Hickman, M.J., Sodergren, E., Weinstock, G.M., Botstein, D., and Desai, M.M. (2013). Pervasive genetic hitchhiking and clonal interference in forty evolving yeast populations. Nature *500*, 571–574.
6. Bock, D.G., Andrew, R.L., and Rieseberg, L.H. (2014). On the adaptive value of cytoplasmic genomes in plants. Mol. Ecol. *23*, 4899–4911.
7. Wright, S.I., Nano, N., Foxe, J.P., and Dar, V.-U.N. (2008). Effective population size and tests of neutrality at cytoplasmic genes in *Arabidopsis*. Genet. Res. *90*, 119–128.
8. Charlesworth, D., and Wright, S.I. (2001). Breeding systems and genome evolution. Curr. Opin. Genet. Dev. *11*, 685–690.
9. Gronwald, J.W. (1994). Resistance to photosystem II inhibiting herbicides. In Herbicide Resistance in Plants: Biology and Biochemistry, S.B. Powles, and J.A. Holtum, eds. (Lewis Publishers), pp. 27–60.
10. Heap, I. (2015). International Survey of Herbicide Resistant Weeds. www.weedscience.com.

11. Darmency, H. (2013). Pleiotropic effects of herbicide-resistance genes on crop yield: a review. Pest Manag. Sci. *69*, 897–904.
12. Arntz, A.M., DeLucia, E.H., and Jordan, N. (2000). From fluorescence to fitness: variation in photosynthetic rate affects fecundity and suvivorship. Ecology *81*, 2567–2576.
13. Warwick, S.I., and Black, L.D. (1994). Relative fitness of herbicide-resistant and susceptible biotypes of weeds. Phytoprotection *75*, 37–49.
14. El-Lithy, M.E., Rodrigues, G.C., van Rensen, J.J.S., Snel, J.F.H., Dassen, H.J.H.A., Koornneef, M., Jansen, M.A.K., Aarts, M.G.M., and Vreugdenhil, D. (2005). Altered photosynthetic performance of a natural *Arabidopsis* accession is associated with atrazine resistance. J. Exp. Bot. *56*, 1625–1634.
15. Platt, A., Horton, M., Huang, Y.S., Li, Y., Anastasio, A.E., Mulyati, N.W., Ågren, J., Bossdorf, O., Byers, D., Donohue, K., et al. (2010). The scale of population structure in *Arabidopsis thaliana*. PLoS Genet. *6*, e1000843.
16. Blanchet, É., Penone, C., Maurel, N., Billot, C., Rivallan, R., Risterucci, A.-M., Maurice, S., Justy, F., Machon, N., and Noël, F. (2015). Multivariate analysis of polyploid data reveals the role of railways in the spread of the invasive South African Ragwort (*Senecio inaequidens*). Conserv. Genet. *16*, 523–533.
17. Network Rail (2015). www.networkrail.co.uk.
18. Palumbi, S.R. (2001). Humans as the world's greatest evolutionary force. Science *293*, 1786–1790.
19. Flood, P.J., Harbinson, J., and Aarts, M.G.M. (2011). Natural genetic variation in plant photosynthesis. Trends Plant Sci. *16*, 327–335.
20. Fatichi, S., Leuzinger, S., and Körner, C. (2014). Moving beyond photosynthesis: from carbon source to sink-driven vegetation modeling. New Phytol. *201*, 1086–1095.
21. Plowman, A.B., Richards, A.J., and Tremayne, M.A. (1999). Environmental effects on the fitness of triazine-resistant and triazine-susceptible *Brassica rapa* and *Chenopodium album* in the absence of herbicide. New Phytol. *141*, 471–485.
22. Gassmann, A.J., and Futuyma, D.J. (2005). Consequence of herbivory for the fitness cost of herbicide resistance: photosynthetic variation in the context of plant-herbivore interactions. J. Evol. Biol. *18*, 447–454.
23. Salzmann, D., Handley, R.J., and Müller-Schärer, H. (2008). Functional significance of triazine-herbicide resistance in defence of *Senecio vulgaris* against a rust fungus. Basic Appl. Ecol. *9*, 577–587.
24. Falahati-Anbaran, M., Lundemo, S., and Stenøien, H.K. (2014). Seed dispersal in time can counteract the effect of gene flow between natural populations of *Arabidopsis thaliana*. New Phytol. *202*, 1043–1054.
25. Lundemo, S., Falahati-Anbaran, M., and Stenøien, H.K. (2009). Seed banks cause elevated generation times and effective population sizes of *Arabidopsis thaliana* in northern Europe. Mol. Ecol. *18*, 2798–2811.
26. Banks, S.C., Cary, G.J., Smith, A.L., Davies, I.D., Driscoll, D.A., Gill, A.M., Lindenmayer, D.B., and Peakall, R. (2013). How does ecological disturbance influence genetic diversity? Trends Ecol. Evol. *28*, 670–679.
27. Corbett-Detig, R.B., Hartl, D.L., and Sackton, T.B. (2015). Natural selection constrains neutral diversity across a wide range of species. PLoS Biol. *13*, e1002112.
28. Wijnker, E., van Dun, K., de Snoo, C.B., Lelivelt, C.L., Keurentjes, J.J., Naharudin, N.S., Ravi, M., Chan, S.W., de Jong, H., and Dirks, R. (2012). Reverse breeding in *Arabidopsis thaliana* generates homozygous parental lines from a heterozygous plant. Nat. Genet. *44*, 467–470.
29. Baker, N.R. (2008). Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu. Rev. Plant Biol. *59*, 89–113.