

INVITED REVIEWS AND SYNTHESSES

On the adaptive value of cytoplasmic genomes in plants

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Abstract

Is DNA variation maintained in organelle genomes selectively neutral? The answer to this question has important implications for many aspects of ecology and evolution. While traditionally the answer has been ‘yes’, recent studies in animals have shown that, on the contrary, mitochondrial DNA polymorphism is frequently adaptive. In plants, however, the neutrality assumption has not been strongly challenged. Here, we begin with a critical evaluation of arguments in favour of this long-held view. We then discuss the latest empirical evidence for the opposing prediction that sequence variation in plant cytoplasmic genomes is frequently adaptive. While outstanding research progress is being made towards understanding this fundamental topic, we highlight the need for studies that combine information ranging from field experiments to physiology to molecular evolutionary biology. Such an interdisciplinary approach provides a means for determining the frequency, drivers and evolutionary significance of adaptive organelle DNA variation.

Keywords: chloroplast DNA, mitochondrial DNA, neutrality tests, plant local adaptation, positive Darwinian selection, selective neutrality

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Is organelle DNA variation neutral?

For more than four decades, phylogenetic and phylogeographic studies in animals and plants have relied heavily on variation in organellar genomes (Avice *et al.* 1979; Scowcroft 1979), with the assumption that sequence polymorphism maintained at the level of the plasmotype (see Glossary 1) is selectively neutral. This assumption is based to a large extent on the fact that organelle genes have repeatedly been shown to evolve under strong purifying selection (Box 1), and—in agreement with the neutral theory of molecular evolution (Kimura 1983)—under such conditions, the fate of persisting variation should be dominated by genetic drift. While purifying selection does not exclude the possibility of positive selection, neutrality conditions for organelle DNA variation are often implicitly assumed to be met.

Studies in animals have increasingly contested the generality of this assumption (Ballard & Whitlock 2004;

Dowling *et al.* 2008; Galtier *et al.* 2009; Balloux 2010). First, parallels have been reported between mitochondrial DNA (mtDNA) haplotype frequencies and natural or laboratory-manipulated conditions (reviewed in Toews & Brelsford 2012). Second, functional differences with potential fitness implications have been assigned to naturally occurring mtDNA variants (reviewed in Ballard & Melvin 2010; Toews *et al.* 2013). Third, the signs of positive selection at mtDNA have been detected using neutrality tests (Ruiz-Pesini *et al.* 2004; Bazin *et al.* 2006; da Fonseca *et al.* 2008; Llopart *et al.* 2014).

With some exceptions (Budar & Roux 2011; Greiner & Bock 2013), the plant literature remained relatively quiescent to these developments. Assessing the validity of the neutrality assumption for plant organelle genetic variation is opportune for two reasons. First, lessons from plant physiology teach us that if organelle genomes are under positive selection, organelle-encoded adaptations are likely to be involved in traits of major conservation, ecological and economical importance, especially in the context of accelerating climate warming, such as tolerance to drought, light and salt

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Box 1. Purifying selection in organelle genomes

Mitochondria and chloroplasts are firmly positioned at the hub of cellular metabolism. The critical importance of both organelles and the genes they retain has been confirmed repeatedly by observations that organelle malfunction and minute changes at organelle DNA can have severely debilitating consequences and may even culminate in lethality (Wallace 2005; Greiner 2012). These and other observations, such as the fact that organelle genomes are highly conserved in structure, have contributed to the view that purifying selection is the predominant force shaping organelle DNA evolution.

This assumption has been substantiated by multiple lines of evidence. For one, early comparisons of rates of synonymous and nonsynonymous substitution at organelle DNA reported an excess of nonsynonymous changes within species, as compared to those detected between species (Nachman 1998; Rand & Kann 1998). This indicated that many organelle DNA nonsynonymous mutations that contribute to intraspecific polymorphism are evolutionarily ephemeral and removed by purifying selection before they can accumulate as interspecific divergences.

Consistent results were provided by empirical studies. For example, in the near absence of natural selection, mutation accumulation experiments reported a considerable increase in the number of nonsynonymous mutations in mtDNA as compared to estimates obtained using phylogenetic comparisons of species pairs (Haag-Liautard *et al.* 2008). Also, studies using mice mutator lines, which express a proofreading-deficient mitochondrial DNA polymerase, revealed that over the course of only two generations, a large proportion of nonsynonymous changes in organelle protein-coding genes are eliminated (Stewart *et al.* 2008). Recent evidence from *Drosophila* suggests this rapid purging is achieved *via* selection at the organelle level, through the preferential propagation of unimpaired haplotypes during oogenesis (Hill *et al.* 2014).

In plants, examples analogous to mutation accumulation experiments can be observed in the wild, in species that have made the evolutionary leap to parasitism. By relying on their hosts for nutrient and carbon uptake, parasitic plants have partially or completely shed the need to fix carbon autotrophically *via* photosynthesis, thereby loosening the selective clench on chloroplast genome variation (Krause 2012). Studies of organelle genome evolution in parasitic plants report extensive genome rearrangements, gene losses and increased rates of base substitution (Krause 2012). These results are consistent with evolution under relaxed purifying selection, although other factors, including positive selection, reduced effective population sizes or increased mutation rates, also appear to be at least partially implicated (Bromham *et al.* 2013).

stress (Atkin & Macherel 2009; Chaves *et al.* 2009). Second, advances in sequencing technology have contributed to increased interest in using complete organelle genomes in studies of phylogenetics, phylogeography and population genetics (Straub *et al.* 2012; Bock *et al.* 2014; Mariac *et al.* 2014). Non-neutrality of organelle DNA polymorphism might, in this case, have important bearing on our ability to infer evolutionary processes from patterns of genomic variation.

We begin this review with a critical evaluation of arguments at the centre of the assumption that plant organelle DNA variation is neutral. We then discuss why theory predicts adaptive evolution at plant organelle DNA is possible, and highlight the strengths and limitations of the latest supporting empirical evidence. Some aspects of our argumentation are necessarily based on a limited number of examples currently available. Nevertheless, we hope information presented here can serve as an impetus for future studies aiming to advance our understanding of this fundamental topic further.

Arguments for the neutrality of plant organelle DNA variation

Neutralist interpretations for plant organelle genetic variation can be traced to a series of three arguments. While the first two of these are shared with the animal mitochondrial genome, the third is applicable to organelle genetic variation in plants.

Argument (i): nonsynonymous DNA polymorphism should be rare in organelle genomes

According to this argument, chloroplast and mitochondrial genomes are unlikely to undergo adaptive evolution, as they should retain limited amounts of nonsynonymous DNA polymorphism within populations (Dowling *et al.* 2008; Galtier *et al.* 2009; Budar & Roux 2011). This is because organelle genes are under strong purifying selection (Box 1), and nonsynonymous mutations that occur in a haploid genome should be continuously exposed to selection.

On careful examination, it is clear that this argument does not fully consider the biology of cytoplasmic genomes and that other attributes of plastomes and chondriomes suggest there should be scope for nonsynonymous organelle DNA variation. For example, on account of haploidy and generally uniparental inheritance, the effective population size of organelle genomes is reduced relative to that of the nuclear genome (Birky *et al.* 1983; Dowling *et al.* 2008). While low effective population size is often associated with a reduction in genetic diversity (Cutter & Payseur 2013), it is also predicted to reduce the efficiency of selection. Moreover, because of complete linkage, selective interference (Hill & Robertson 1966) should be high in organelle DNA. From this perspective, organelle genomes should behave similarly to regions of the nuclear genome that have a long history of reduced recombination, such as the dot chromosome in *Drosophila*, or the degenerate sex chromosomes of dioecious animals and plants, which show accelerated accumulation of nonsynonymous polymorphisms (Betancourt *et al.* 2009; Hough *et al.* 2014).

Empirical evidence supports these predictions. One example is the study by Drouin *et al.* (2008). The authors surveyed the rates of synonymous and nonsynonymous substitutions in three mitochondrial, five chloroplast, and four nuclear genes for 27 seed plant species. While rates of nonsynonymous polymorphisms were 46 times lower than rates of synonymous polymorphisms for nuclear genes, these differences were considerably reduced for organelle genes. Specifically, nonsynonymous rates estimated at 0.042 substitutions per site for mitochondrial genes and at 0.082 substitutions per site for chloroplast genes were only six and seven times lower than synonymous rates inferred for the same loci (Drouin *et al.* 2008).

Argument (ii): organelle genomes have limited coding potential

According to this argument, because mitochondria and chloroplasts relinquished most of their genes to the nuclear genome during endosymbiotic gene transfer (Timmis *et al.* 2004), most organelle functions are under nuclear control. Therefore, even if local adaptation requires changes in organelle function, these are most likely to be encoded in nuclear DNA.

Of course, we already know that the apparent simplicity of organelle genomes cannot be taken as *prima facie* evidence for the adaptive neutrality of organelle DNA variation. A number of adaptive responses have been traced back to the compact mitochondrial and chloroplast genomes of animals and plants. In animals, for which the coding capacity of the chondriome is even

more reduced than it is in plants (Timmis *et al.* 2004), naturally occurring mtDNA variants have been shown to differentially affect a host of traits including lifespan, fecundity or starvation resistance (reviewed in Ballard & Melvin 2010; Toews *et al.* 2013). In plants, the case of weed resistance to triazine herbicides constitutes a textbook example. Over the past 40 years, persistent application of triazine herbicides has imposed a strong selective pressure for the evolution of resistance on weed populations globally (Powles & Yu 2010). As it was initially reported in the 1970s, triazine resistance has been described in at least 68 weed species (Powles & Yu 2010). In the majority of these cases, the resistance trait has been mapped to a point mutation in the plastome *psbA* gene (Powles & Yu 2010). Another compelling example from plants is chilling tolerance in cucumber. Gordon & Staub (2011) used reciprocal backcrosses between chilling-sensitive and chilling-tolerant lines to show that tolerance to reduced temperature is inherited maternally, with the nuclear genome having a negligible contribution. The causative mutations for this trait are most likely located in the chloroplast genome, as only the plastome is inherited maternally in cucumber, while the chondriome is inherited paternally (Gordon & Staub 2011). This possibility is reinforced by the fact that strong associations between three single nucleotide polymorphisms (SNPs) in the cucumber plastome and chilling tolerance have been reported previously (Chung *et al.* 2007).

Argument (iii): mutation rates are reduced for plant organelle DNA

Contrary to animal mtDNA, plant organelle DNA often shows markedly reduced mutation rates (see Box 2 for a description of patterns reported as well as mechanistic explanations). Because the rate of adaptation is limited by the supply of mutations, a third argument that can be made is that low mutational input limits adaptive evolution of plant organelle genomes.

There are three caveats to this argument. First, while mutation rates will determine the amount of standing or *de novo* variation available for adaptive evolution, we know that a third source of variation, introgression, is common for plant organelle genomes (Rieseberg & Soltis 1991). Indeed, evidence has been provided for trans-species selective sweeps at plant organelle DNA (see 'Observational evidence' below; Muir & Filatov 2007). Second, we do not know how much organelle DNA variation is needed for an adaptive response under changing environments. The examples of resistance to triazine herbicides in weeds or chilling tolerance in cucumber (Powles & Yu 2010; Gordon & Staub 2011) suggest the slightest alterations in organelle DNA can

Box 2. Variation in organelle DNA mutation rates

While in animals, mtDNA mutation rates are 5–50 times faster than for nuclear DNA (Brown *et al.* 1979), the situation is often inversed in plants. In a pioneering study 27 years ago, Wolfe *et al.* (1987) showed that genes in the mitochondrial and chloroplast genomes of plants evolve at approximately sixfold and twofold slower rates, respectively, than genes in the nuclear genome.

More recent studies have made use of an increasing amount of DNA sequence data to further revise these estimates. Drouin *et al.* (2008), for example, documented levels of polymorphism retained in three mitochondrial, five chloroplast and four nuclear genes for 27 seed plant species. Results confirmed the patterns initially reported by Wolfe *et al.* (1987), although the magnitude of differences was shown to differ between plant groups. For instance, the ratios of mitochondrial to chloroplast to nuclear DNA synonymous substitutions were estimated to be 1:3:16 for angiosperms, compared to 1:2:4 for gymnosperms (Drouin *et al.* 2008). Consistent with the view that organelle DNA evolves mainly under purifying selection (Box 1), the rates of nonsynonymous substitution for the same organelle genes and taxa were 6–7 times lower than rates of synonymous substitution (Drouin *et al.* 2008).

Mechanistic explanations for the discrepancies between animal and plant systems in organelle DNA mutation rates have generally revolved around differences that exist between animals and plants in the nuclear-encoded machinery of organelle DNA replication and repair. For instance, high mutation rates in animal mtDNA have been suggested to be at least partially caused by the absence, in animal nuclear genomes sequenced to date, of homologs for the *mutS* and *recA* genes (Lin *et al.* 2006; Sloan & Taylor 2012). Both of these are classic players in bacterial DNA recombination and mismatch repair that seem to have been lost in animals during endosymbiotic gene transfers (Lin *et al.* 2006; Sloan & Taylor 2012).

In plants on the other hand, homologs for *mutS* and *recA* are present in multiple active copies (e.g. Lin *et al.* 2006; Maréchal & Brisson 2010). Studies using mutants and RNA interference have illustrated that products of these genes limit the frequency of illegitimate recombination and genome rearrangements in the plastome and chondriome (Maréchal & Brisson 2010; Sloan & Taylor 2012). Given that gene conversion, a process relying on recombination, has been shown experimentally to contribute to the elimination of *de novo* base pair substitutions in tobacco plastomes (Khakhlova & Bock 2006), it is likely that a similar mechanism also contributes to the maintenance of reduced point mutation rates at plant organelle genomes.

have important adaptive consequences. Third, comprehensive analyses of plant organelle DNA variation have been uncommon until recently, and, with some exceptions, low mutation rates have been substantiated by surveys of few genes and/or a limited number of samples. With the accumulation of DNA sequence over the past decade, mutation rate speed-ups have been described at multiple levels of biological organization (Table 1), and the generality of this assumption has been shaken. Thus, it is not reasonable to attribute low adaptability of plant organelle DNA to its universally low rate of mutation.

Evidence for an adaptive value of plant cytoplasm

The arguments outlined above, while firmly grounded in organelle biology, then do not seem to exclude the possibility that positive selection may shape plant organelle DNA diversity. So is there evidence that plant organelle genetic variation is adaptive?

Characteristics of plant dispersal and gene flow suggest the maternal contribution of the genome should be

a prime target for adaptive divergence. Plant dispersal is mediated by pollen and seed. Among these, seed is known to have a disproportionately lower contribution to dispersal (Petit *et al.* 2005). In agreement with this observation, maternally inherited markers show more subdivision among plant populations than paternally or biparentally inherited ones (Petit *et al.* 2005). Chloroplasts may be inherited paternally, as in conifers (Neale *et al.* 1986), or biparentally, as in *Passiflora* (Hansen *et al.* 2007), and patterns of mitochondrial variation in some cases suggest occasional leakage and recombination of paternal mitochondrial genomes (Jaramillo-Correa & Bousquet 2005; McCauley 2013). Nevertheless, inheritance of organelles is overwhelmingly uniparental and typically maternal in most groups. Given that the diversifying effects of positive selection are hindered by the homogenizing effects of gene flow, high genetic subdivision of maternally inherited genomes may mean that even weak selection at plant organelle DNA can be sufficient to drive local adaptation.

Computer simulations support this argument. Irwin (2012) used individual-based modelling to track the genealogy of a uniparentally inherited locus and the

Table 1 Examples of studies reporting accelerated rates of nucleotide substitution in plant organelle genomes

Taxa investigated	Regions with accelerated rates in one or more taxa	<i>dN</i> elevated	<i>dS</i> elevated	References
Flowering plants (50 taxa)	C: <i>atp1</i> , <i>cob</i> , <i>cox1</i> , <i>cox2</i> , <i>LSU rDNA</i> , <i>SSU rDNA</i>	•	•	Cho <i>et al.</i> (2004)
Flowering plants (58 taxa)	C: <i>atp1</i> , <i>cob</i> , <i>cox1</i> , <i>cox2</i> , <i>cox3</i> , <i>nad1</i> , <i>SSU rDNA</i> , <i>LSU rDNA</i>	•	•	Parkinson <i>et al.</i> (2005)
Flowering plants (127 taxa)	C: <i>nad1</i>		•	Bakker <i>et al.</i> (2006)
<i>Silene vulgaris</i> (25 samples)	C: <i>atp1</i>		•	Barr <i>et al.</i> (2007)
Land plants (306–578 taxa, depending on gene used)	C: <i>atp1</i> , <i>cob</i> , <i>cox1</i> , <i>cox2</i> , <i>cox3</i> , <i>matR</i> , <i>LSU rDNA</i> , <i>SSU rDNA</i>	•	•	Mower <i>et al.</i> (2007)
<i>Sileneae</i> (21 taxa) and <i>Oenothera</i> (4 taxa)	P: <i>clpP1</i>	•	•	Erixon & Oxelman (2008)
Flowering plants (47 taxa)	P: <i>rpl</i> , <i>rps</i> , <i>rpo</i> , <i>psb</i> genes	•	•	Guisinger <i>et al.</i> (2008)
<i>Silene</i> (4 species)	C: complete genomes	•	•	Sloan <i>et al.</i> (2012)
<i>Pelargonium</i> (58 species)	P: <i>rpoC1</i> C: <i>nad5</i>	•	•	Weng <i>et al.</i> (2012)
<i>Sileneae</i> (7 species)	P: <i>clpP</i> , <i>ycf1</i> , <i>ycf2</i>	•	•	Sloan <i>et al.</i> (2014a)
Geraniales (11 species)	P: complete genomes	•		Weng <i>et al.</i> (2014)
<i>Ajuga reptans</i>	C: <i>atp9</i> , <i>rps3</i> , <i>rps12</i> P: <i>atpH</i>	•	•	Zhu <i>et al.</i> (2014)

'P' is used to indicate loci in the plastome, and 'C' is used to indicate loci in the chondriome.

distribution of the phenotypic values linked to it, under spatially varying selection. Under conditions of high dispersal and extremely weak selection, the locus behaved neutrally. When dispersal was moderate, however, even fairly weak selection led to the formation of locally adapted clades (Irwin 2012). We next discuss empirical evidence that supports this theoretical prediction, pointing to adaptive plant organelle DNA variation.

Observational evidence: studies of organelle genome capture

The replacement of one species' or population's organelle genomes with those of another has been observed in a range of taxa (Rieseberg & Soltis 1991). Commonly referred to as chloroplast or mitochondrial capture, this phenomenon is thought to originate by hybridization followed by repeated backcrossing to the pollen donor or *via* asexual transfer of organelles across natural grafts (Stegemann *et al.* 2012; Fuentes *et al.* 2014).

While the mechanisms by which capture takes place are clear, the evolutionary contexts it occurs in are less well understood. A common interpretation is that these events are selectively neutral, resulting from incomplete lineage sorting (Comes & Abbott 2001), from stochastic surfing of alien cytoplasm during range expansions (Neiva *et al.* 2010), from differential allocation to female reproductive functions (Tsitrone *et al.* 2003) or because reproductive barriers between species are asymmetric (McKinnon *et al.* 2004). Another possibility is that organelle capture events are adaptive, with plasmotypes being transferred because they confer a selective

advantage (Toews & Brelsford 2012; Greiner & Bock 2013). This scenario is used to interpret cases where captured haplotypes are associated with geography. Examples of such associations abound in animals (Toews & Brelsford 2012). In plants, however, they are less frequently reported, potentially because landscape-level surveys of organelle DNA variation have been relatively rare in plants (Schaal *et al.* 1998).

Some of the best known plant examples of chloroplast introgression come from studies of European white oaks. Petit *et al.* (2002a) performed what is to date one of the most ambitious landscape-level surveys of plant organelle DNA variation. The authors sampled over 2600 European populations of eight white oak species and typed 12 214 individuals at chloroplast DNA. Chloroplast capture was inferred to be extensive, as haplotypes did not group by species. Instead, six chloroplast DNA clades were distributed along a longitudinal gradient across the continent (Petit *et al.* 2002a). This result built on previous findings in white oaks of such associations at the regional and local scales as well (Dumolin-Lapegue *et al.* 1997; Petit *et al.* 1997, 2002b). Other similar examples have since been provided by studies in European *Betula* (Palme *et al.* 2004) or South American *Nothofagus* (Acosta & Premoli 2010).

On a cursory examination, it seems reasonable to assume that positive selection was involved. Transferred haplotypes could be more fit if, for example, they are less mutationally loaded than haplotypes being replaced, or they could be better adapted to local environments. Although intuitively appealing, this adaptive designation is premature, as alternative neutral

scenarios can generate similar patterns. For example, it may be that the front of organelle DNA introgression is moving and overlaps with environment by chance. Indeed, the dominant interpretation of these data has been adaptively neutral, with introgression driven by invasion of the pollen parent (e.g. Petit *et al.* 2004). In the light of the growing evidence for adaptive evolution of organelle genomes, additional analyses are required to identify determinants of organelle capture.

Scenarios of neutral and adaptive organelle introgression can be tested by looking for the DNA footprints of positive selection. For example, neutral population growth and positive selection are both expected to lead to an excess of rare polymorphisms. One can differentiate between the two scenarios using coalescent simulations, by comparing levels of observed variation with those expected under neutrality (Llopart *et al.* 2014). Alternatively, tests of neutrality can be applied concomitantly to organelle and nuclear DNA data. Contrary to the signature for positive selection, which should be found only in organelle DNA if capture events are adaptive, neutral factors such as population expansion should leave a trace in both organelle and nuclear DNA.

This approach was used by Muir & Filatov (2007). The authors sampled populations of the hybridizing angiosperm species *Silene latifolia* and *S. dioica* across Eurasia, and typed specimens at organelle and nuclear DNA. Consistent with chloroplast capture, there was extensive haplotype sharing between species in regions of range overlap. Also, analyses rejected neutrality for chloroplast genes. By contrasting these results with those obtained for nuclear DNA, which behaved according to neutral expectations, the authors were able to exclude the possibility that organelle capture was neutral (Muir & Filatov 2007). Instead, a selective sweep was inferred to have occurred in the *Silene* plastome between 0.16 and 1.06 Ma, which then crossed the *Silene* species boundaries (Muir & Filatov 2007).

Experimental evidence: studies of cytonuclear interactions

Strong evidence for local cytoplasmic adaptation has been provided by studies aiming to understand the determinants of cytonuclear interactions (CNI) (Burton *et al.* 2013). These studies use crosses or *in vitro* manipulation to make lines for which the native plasmotype has been replaced with the plasmotype of a different species or ecotype (Burton *et al.* 2013; Greiner & Bock 2013).

The fitness of such alloplasmic lines can be reduced irrespective of environment. In this case, intrinsic selection is thought to act against dissonant interactions between nuclear and organelle genomes that are not adapted to function in the same cell (Burton *et al.* 2013;

Greiner & Bock 2013). Occasionally, genes involved in these interactions have been identified (Maheshwari & Barbash 2011). For example, the albino phenotype of hybrids carrying the chloroplast genome of tobacco and the nuclear genome of deadly nightshade was shown to result from defective RNA editing of the tobacco plastid *atpA* gene by nightshade nuclear-encoded enzymes (Schmitz-Linneweber *et al.* 2005). If, however, the fitness of alloplasmic lines is contingent on environment, extrinsic ecological selection is inferred to contribute to CNI (Burton *et al.* 2013; Greiner & Bock 2013). Under this scenario, CNI result because organelle genes are locally adapted and potentially also involved in maladaptive crosstalk with nuclear genes, either directly or through linkage.

Sambatti *et al.* (2008) is a well-known example of studies in this category. The authors investigated the contribution of extrinsic ecological selection to CNI between *Helianthus petiolaris* and *H. annuus*, two hybridizing annual sunflowers that occupy contiguous and contrasting habitats in North America (Fig. 1a). The authors carried out a reciprocal transplant experiment using 5600 seedlings of the two species, their reciprocal F1s and eight backcross combinations of nuclear and cytoplasmic genomes (Fig. 1b). Analysis of the survivorship of transplanted genotypes revealed a significant interaction between habitat and the fraction of *H. annuus* nuclear genome, as well as between habitat and the plasmotype of both species (Fig. 1c). These results are a strong indication that ecological differentiation (e.g. drought adaptation) in *H. petiolaris* and *H. annuus* is underpinned not only by nuclear genes, but also by organelle genes (Sambatti *et al.* 2008). Reciprocal transplant experiments have since shown that environment-dependent selection on the cytoplasm contributes to CNI between *Ipomopsis aggregata* and *I. tenuituba* (Campbell *et al.* 2008), and between *Penstemon newberryi* and *P. davidsonii* (Kimball *et al.* 2008), two pairs of species that hybridize along altitudinal clines.

Compelling examples exist at the infraspecific level as well. Leinonen *et al.* (2011), for instance, performed a reciprocal transplant of *Arabidopsis lyrata* subspecies that diverged in allopatry in Europe and North America, as well as their F1 and F2 reciprocal hybrids. As expected if cytoplasmic genomes—either alone or *via* their interaction with the nuclear genome—contribute to local adaptation, a strong positive effect on fitness was observed for the local cytoplasm (Leinonen *et al.* 2011). This pattern was pursued further in a follow-up study that used quantitative trait locus (QTL) mapping to understand the number and genomic location of nuclear genes that interact with cytoplasmic genomes during local adaptation (Leinonen *et al.* 2013). A fitness advantage of local nuclear alleles was associated with the local cytoplasm only at some QTLs, and only in

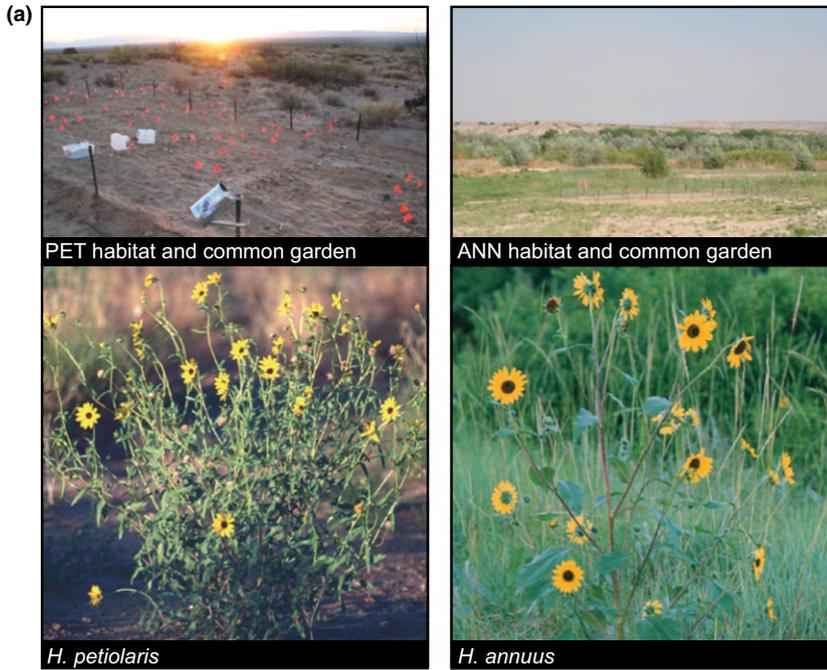
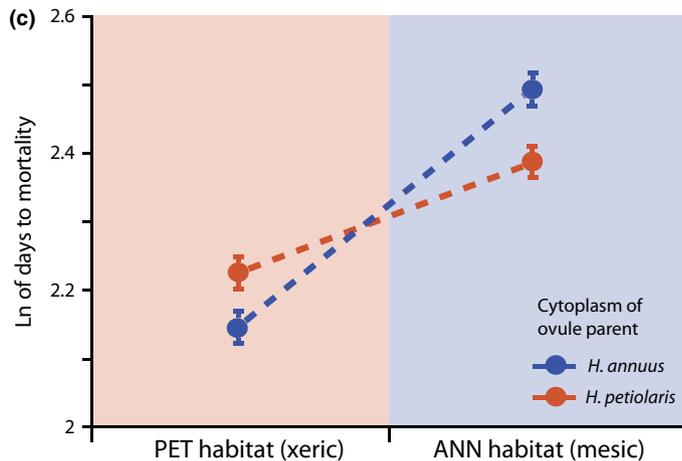
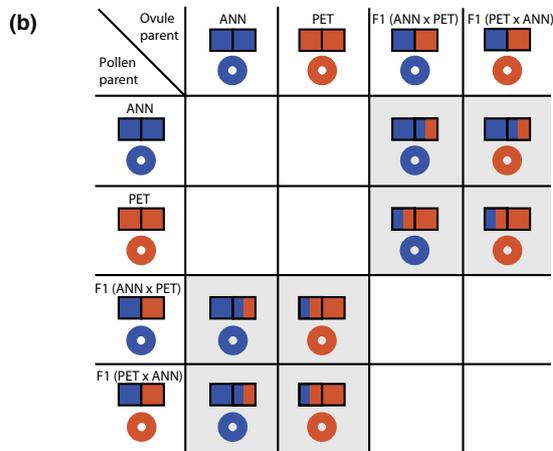


Fig. 1 Example of experimental confirmation of the adaptive contribution of the plant plasmotype. (a) *Helianthus petiolaris* (PET), *H. annuus* (ANN) and common garden locations used in Sambatti *et al.* (2008). (b) Crosses used in Sambatti *et al.* (2008) to obtain different nuclear genome—organelle genome combinations. For the F1s, the maternal parent is listed first. The eight possible backcross combinations are indicated with grey shading. Squares represent the nuclear genomes, while open circles represent the plasmotype for PET (red) and ANN (blue). (c) Cytoplasm by habitat interaction for survivorship expressed as the mean \pm SE of the natural log of days to mortality for individuals sharing the same cytoplasm (reproduced with permission from Sambatti *et al.* (2008); photo credits: JBM Sambatti, GJ Seiler, J Rick).



European samples. These results showed that fitness advantages of local cytoplasm observed by Leinonen *et al.* (2011) are largely conferred by variation in organelle genomes, and not by CNIs (Leinonen *et al.* 2013).

Studies such as those outlined above, which assess the fitness of experimental crosses under natural settings, are a powerful way of determining whether plant cytoplasm contributes to local adaptation. One limitation of this approach is that, unless environmental variables are experimentally manipulated, it does not by itself provide any measure of the selection pressures that may be driving ecological divergence. Also, unless used in species for which the plastome and chondriome have opposite modes of inheritance, the experimental approach does not allow inferences to be made regarding which of the two organelle genomes is the target of selection.

Statistical evidence: studies of positive selection at the molecular level

The adaptive contribution of plant organelle genetic variation has also been studied by looking for footprints left by positive selection in patterns of DNA variation. One of the first and most taxonomically diverse studies in this category is that by Kapralov & Filatov (2007). The authors leveraged the wealth of sequence data generated for phylogenetic purposes for the plastome *rbcl* gene, which encodes the large subunit of the photosynthetic enzyme Rubisco. Their data set included 3228 sequences obtained from all lineages of green plants, and some lineages of brown and red algae, diatoms, euglenids and cyanobacteria. Contrary to the traditional view that plant organelle DNA variation is neutral, the *dN/dS* ratio test provided evidence for positive selection at *rbcl* in as many as 75–88% of land plants (Kapralov & Filatov 2007). This result was followed by a number of other similar studies. While some provided results consistent with neutral organelle polymorphism (e.g. Wright *et al.* 2008), others reported patterns indicative of non-neutrality (Table 2). Similarly to the Kapralov & Filatov (2007) example, many of these used the *dN/dS* ratio test to look for sites under positive selection along a gene of interest and across a phylogeny (Table 2).

Loci involved in local adaptation can also be identified by searching for correlations between allele frequencies and environmental variables (Coop *et al.* 2010). This is a powerful way to study adaptive organelle DNA evolution, as it allows inferences to be made not only on the genetic basis of local adaptation, but also on likely agents of selection. Ideally, to understand the full genetic architecture of adaptive responses, genome-wide surveys of polymorphism should be performed. A less comprehensive but still valuable approach is to rely on knowledge of gene function and to select a subset of

loci suspected to be involved in the adaptations of interest. One example is the study of Bashalkhanov *et al.* (2013). The authors performed an environmental correlation analysis in red spruce, using SNPs from 36 nuclear and plastome candidate genes, chosen for their likely involvement in adaptation to climate and human-induced air pollution. Polymorphism at six nuclear genes, as well as the plastome *chlB* gene, which encodes for the light-independent protochlorophyllide reductase, was strongly associated with 19 climatic variables, suggesting these loci have been targets of spatially variable selection (Bashalkhanov *et al.* 2013).

Ideally, statistical inferences of positive selection should be interpreted in conjunction with experimental evidence. This is because false-positive rates of neutrality tests can be high if the underlying demographic assumptions are unrealistic (Nielsen 2001; Beaumont & Balding 2004; Beaumont 2005). Moreover, interdisciplinary approaches are more likely to paint a complete picture of the genetic and ecological contexts of adaptive evolution. Galmes *et al.* (2014) used this strategy to investigate whether positive selection at the plastome *rbcl* gene contributed to adaptation to drought conditions during the recent diversification of the perennial angiosperm genus *Limonium* in the Balearic Islands. Two derived substitutions at functionally important Rubisco residues, I309M and S328A, were inferred to have been the result of positive selection according to the *dN/dS* ratio test. *In vitro* enzymatic assays confirmed that these substitutions are associated with increased CO₂ affinity and reduced carboxylase efficiency of Rubisco. By rearing plants with both derived and ancestral *rbcl* haplotypes under irrigated and water-limited conditions, the authors were able to identify that the levels of CO₂ available in the chloroplast stroma during periods of drought were the likely selective agent driving these substitutions (Galmes *et al.* 2014).

Neutrality tests can be used to dissect episodes of adaptive evolution at the molecular level. Apart from the scarcity of experimental confirmation of putative examples of molecular adaptation, the greatest limitation of studies in this category performed so far is that they have relied on a limited number of genes. Such approaches may allow, at best, only incomplete glimpses of non-neutrality of organelle DNA variation. Analyses of complete or nearly complete organelle genomes, which have now become increasingly accessible, should provide a more unbiased look at positive selection at the level of the plastome.

Future directions

In this review, we presented experimental evidence for why it should no longer be assumed that plant organelle

Table 2 Examples of studies using neutrality tests of DNA polymorphism to infer positive selection at organelle genes. All loci investigated were located in the plastome

Taxa included in analysis	Genes investigated	Neutrality test used	Selected genes	Putative agent of selection	References
<i>Schiedea</i> (27 taxa)	<i>matK, psbA, rbcL</i>	<i>dN/dS</i> ratio test	<i>rbcL</i>	Photosynthetic performance under dry sunny conditions	Kapralov & Filatov (2006)
Green plants, brown and red algae, diatoms, euglenids and cyanobacteria (3228 taxa)	<i>rbcL</i>	<i>dN/dS</i> ratio test	<i>rbcL</i>	Photosynthetic performance under fluctuating thermal and gaseous conditions in terrestrial environments	Kapralov & Filatov (2007)
<i>Silene latifolia</i> (75 samples) and <i>S. dioica</i> (29 samples)	<i>trnL, matK, rbcL</i>	HKA and Tajima's <i>D</i>	<i>matK, rbcL, trnL + matK</i>	Not discussed	Muir & Filatov (2007)
Commelinoid monocots (338 taxa)	<i>rbcL, ndhF</i>	<i>dN/dS</i> ratio test	<i>rbcL</i>	Photosynthetic performance in CO ₂ -rich bundle sheath cells of C ₄ plants	Christin <i>et al.</i> (2008)
<i>Sileneae</i> (21 taxa) and <i>Oenothera</i> (4 taxa)	<i>clpP1</i>	<i>dN/dS</i> ratio test	<i>clpP1</i>	Not discussed	Erixon & Oxelman (2008)
Flowering plants (47 taxa)	72 plastid genes	<i>dN/dS</i> ratio test	<i>rpoB, rpoC1, rpoC2</i>	Not discussed	Guisinger <i>et al.</i> (2008)
<i>Potamogeton</i> (18 taxa)	<i>rbcL, atpB, petA</i>	<i>dN/dS</i> ratio test	<i>rbcL</i>	Photosynthetic performance under environmental variation in temperature and dryness	Iida <i>et al.</i> (2009)
Green plants (31 taxa)	75 plastid genes	<i>dN/dS</i> ratio test	<i>atpE, cemA, clpP, rpoB, rps11</i>	Not discussed	Zhong <i>et al.</i> (2009)
<i>Pinus</i> (37 taxa)	nearly complete plastomes	<i>dN/dS</i> ratio test	<i>ycf1, ycf2</i>	Not discussed	Parks <i>et al.</i> (2009)
Green plants (2279 taxa)	<i>matK</i>	<i>dN/dS</i> ratio test	<i>matK</i>	Not discussed	Hao <i>et al.</i> (2010)
<i>Flaveria</i> (15 taxa)	<i>ndhF, psbA, rbcL</i>	<i>dN/dS</i> ratio test	<i>rbcL</i>	Not discussed	Kapralov <i>et al.</i> (2011)
Ferns (27 taxa)	<i>psbA</i>	<i>dN/dS</i> ratio test	<i>psbA</i>	Photosynthetic performance under modified light conditions caused by angiosperm diversification	Sen <i>et al.</i> (2011)
<i>Amaranthaceae sensu lato</i> (179 taxa)	<i>rbcL</i>	<i>dN/dS</i> ratio test	<i>rbcL</i>	Photosynthetic performance in warm climates	Kapralov <i>et al.</i> (2012)
<i>Pelargonium</i> (58 species)	<i>rbcL, matK, ndhF, rpoC1, trnL-F</i>	<i>dN/dS</i> ratio test	<i>rpoC1</i>	Not discussed	Weng <i>et al.</i> (2012)
<i>Picea rubens</i>	<i>chlB</i>	Environmental correlation	<i>chlB</i>	Photosynthetic performance under changing climatic conditions	Bashalkhanov <i>et al.</i> (2013)
<i>Sileneae</i> (7 species)	Complete plastomes	<i>dN/dS</i> ratio test	<i>clpP, ycf1, ycf2</i>	Not discussed	Sloan <i>et al.</i> (2014a)
<i>Limonium</i> (42 species)	<i>rbcL</i>	<i>dN/dS</i> ratio test	<i>rbcL</i>	CO ₂ availability under drought conditions	Galmes <i>et al.</i> (2014)

DNA variation is selectively neutral. In doing so, our aim was not to dampen recent excitement about the use of complete sequences of plant organelle genomes in studies of plant phylogenetics and phylogeography. Rather, we hope to caution against the use of these data without testing beforehand that neutrality assumptions are met. This is particularly relevant for studies using population samples, for which neutrality violations are expected to have a disproportionately larger effect. More generally, we aimed to highlight the neglected

possibility that local adaptation of plant populations is underpinned by both nuclear and cytoplasmic genes.

Given that sequence data are being collected at an unprecedented pace, we predict that in the near future, evidence for non-neutrality of plant organelle DNA variation, and in particular that obtained from patterns of organelle capture and tests of neutrality, will continue to accumulate. We can also expect that concomitant improvements in analytical approaches will increase the reliability of inferences of positive selection drawn from

Box 3. Outstanding questions**What is the adaptive value of plant mitochondrial DNA variation?**

Current work on the adaptive contribution of plant organelle genomes has been disproportionately focused on the plastome. This bias is evident when considering studies that used tests of neutrality on organelle genes (Table 2), which have so far only considered loci in the chloroplast genome. There is therefore a need to expand these surveys to the mitochondrial genome. Beyond analyses of DNA polymorphism, substitution crosses and reciprocal transplant experiments should be used in species for which the plastomes and chondriomes have opposite modes of inheritance, to isolate the fitness effects of mitochondrial DNA variation.

What agents of selection cause adaptive evolution at plant organelle DNA?

This topic has attracted surprisingly little explicit interest so far. Most of the information currently available is indirect and stems from knowledge of the ecology of species under investigation (e.g. Sambatti *et al.* 2008) or from correlations between putatively selected haplotypes and environmental variables (e.g. Bashalkhanov *et al.* 2013). Future studies of organelle-encoded fitness effects should aim to test these predictions experimentally, by manipulating biotic and abiotic factors suspected to act as agents of selection. The relationship between agents of selection, the strength of selection and the likelihood of adaptive evolution at organelle DNA should then also be investigated.

Does cytonuclear co-evolution contribute to local adaptation in plants?

Because of their tight functional integration, it has been hypothesized that co-evolution between the nuclear and organelle genomes is common (Burton *et al.* 2013). Examples available so far appear to be cases where deleterious mutations at organelle loci have been selected for compensatory mutations at nuclear loci, to maintain organelle function (Osada & Akashi 2012; Sloan *et al.* 2014b). It is unknown, however, whether such interactions contribute to local adaptation as well. Provided that dense taxonomic sampling is available for organelle and nuclear loci of interest, future studies could investigate this possibility in a phylogenetic framework, to identify which mutations are causal and which mutations are correlated (e.g. Osada & Akashi 2012).

sequence data alone. Even so, by relying on isolated examples obtained from distantly connected branches in the tree of life, we are unlikely to obtain a complete picture of positive selection at plant organelle DNA. This is because answers to many of the currently outstanding questions on this topic, a few of which are highlighted in Box 3, are likely to depend on the species under consideration.

Future approaches should therefore aim to integrate observational, experimental and statistical evidence in multiple systems. Moreover, physiological experiments and functional studies should be implemented to connect molecular and experimental evidence of adaptive evolution with differences in fitness. It is only using an interdisciplinary approach that we can hope to move from documenting isolated examples of adaptive organelle DNA evolution, to understanding its frequency, drivers and evolutionary significance.

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References

- Acosta MC, Premoli AC (2010) Evidence of chloroplast capture in South American *Nothofagus* (subgenus *Nothofagus*, Nothofagaceae). *Molecular Phylogenetics and Evolution*, **54**, 235–242.
- Atkin OK, Macherel D (2009) The crucial role of plant mitochondria in orchestrating drought tolerance. *Annals of Botany*, **103**, 581–597.
- Avisé JC, Giblin-Davidson C, Laerm J, Patton JC, Lansman RA (1979) Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetis*. *Proceedings of the National Academy of Sciences USA*, **76**, 6694–6698.
- Bakker FT, Breman F, Merckx V (2006) DNA sequence evolution in fast evolving mitochondrial DNA *nad1* exons in Geraniaceae and Plantaginaceae. *Taxon*, **55**, 887–896.
- Ballard JWO, Melvin RG (2010) Linking the mitochondrial genotype to the organismal phenotype. *Molecular Ecology*, **19**, 1523–1539.
- Ballard JWO, Whitlock MC (2004) The incomplete natural history of mitochondria. *Molecular Ecology*, **13**, 729–744.
- Balloux F (2010) The worm in the fruit of the mitochondrial DNA tree. *Heredity*, **104**, 419–420.
- Barr CM, Keller SR, Ingvarsson PK, Sloan DB, Taylor DR (2007) Variation in mutation rate and polymorphism among mitochondrial genes in *Silene vulgaris*. *Molecular Biology and Evolution*, **24**, 1783–1791.
- Bashalkhanov S, Eckert AJ, Rajora OP (2013) Genetic signatures of natural selection in response to air pollution in red spruce (*Picea rubens*, Pinaceae). *Molecular Ecology*, **22**, 5877–5889.

- Bazin E, Glémin S, Galtier N (2006) Population size does not influence mitochondrial genetic diversity in animals. *Science*, **312**, 570–571.
- Beaumont MA (2005) Adaptation and speciation: what can Fst tell us? *Trends in Ecology & Evolution*, **20**, 435–440.
- Beaumont MA, Balding DJ (2004) Identifying adaptive genetic divergence among populations from genome scans. *Molecular Ecology*, **13**, 969–980.
- Betancourt AJ, Welch JJ, Charlesworth B (2009) Reduced effectiveness of selection caused by a lack of recombination. *Current Biology*, **19**, 655–660.
- Birky CW Jr, Maruyama T, Fuerst P (1983) An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. *Genetics*, **103**, 513–527.
- Bock DG, Kane NC, Ebert DP, Rieseberg LH (2014) Genome skimming reveals the origin of the Jerusalem Artichoke tuber crop species: neither from Jerusalem nor an artichoke. *New Phytologist*, **201**, 1021–1030.
- Bromham L, Cowman PF, Lanfear R (2013) Parasitic plants have increased rates of molecular evolution across all three genomes. *BMC Evolutionary Biology*, **13**, 126.
- Brown WM, George M Jr, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences USA*, **76**, 1967–1971.
- Budar F, Roux F (2011) The role of organelle genomes in plant adaptation. *Plant Signaling & Behavior*, **6**, 635–639.
- Burton RS, Pereira RJ, Barreto FS (2013) Cytonuclear genomic interactions and hybrid breakdown. *Annual Review of Ecology, Evolution, and Systematics*, **44**, 281–302.
- Campbell DR, Waser NM, Aldridge G, Wu CA (2008) Lifetime fitness in two generations of *Ipomopsis* hybrids. *Evolution*, **62**, 2616–2627.
- Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany*, **103**, 551–560.
- Cho Y, Mower JP, Qiu YL, Palmer JD (2004) Mitochondrial substitution rates are extraordinarily elevated and variable in a genus of flowering plants. *Proceedings of the National Academy of Sciences USA*, **101**, 17741–17746.
- Christin PA, Salamin N, Muasya AM *et al.* (2008) Evolutionary switch and genetic convergence on *rbcl* following the evolution of C4 photosynthesis. *Molecular Biology and Evolution*, **25**, 2361–2368.
- Chung S-M, Gordon VS, Staub JE (2007) Sequencing cucumber (*Cucumis sativus* L.) chloroplast genomes identifies differences between chilling-tolerant and -susceptible cucumber lines. *Genome*, **50**, 215–225.
- Comes HP, Abbott RJ (2001) Molecular phylogeography, reticulation, and lineage sorting in Mediterranean *Senecio* sect. *Senecio* (Asteraceae). *Evolution*, **55**, 1943–1962.
- Coop G, Witonsky D, Di Rienzo A, Pritchard JK (2010) Using environmental correlations to identify loci underlying local adaptation. *Genetics*, **185**, 1411–1423.
- Cutter AD, Payseur BA (2013) Genomic signatures of selection at linked sites: unifying the disparity among species. *Nature Reviews Genetics*, **14**, 262–274.
- Dowling DK, Friberg U, Lindell J (2008) Evolutionary implications of non-neutral mitochondrial genetic variation. *Trends in Ecology & Evolution*, **23**, 546–554.
- Drouin G, Daoud H, Xia J (2008) Relative rates of synonymous substitutions in the mitochondrial, chloroplast and nuclear genomes of seed plants. *Molecular Phylogenetics and Evolution*, **49**, 827–831.
- Dumolin-Lapegue S, Demesure B, Fineschi S, Come VL, Petit RJ (1997) Phylogeographic structure of white oaks throughout the European continent. *Genetics*, **146**, 1475–1487.
- Erixon P, Oxelman B (2008) Whole-gene positive selection, elevated synonymous substitution rates, duplication, and indel evolution of the chloroplast *clpP1* gene. *PLoS One*, **3**, e1386.
- da Fonseca RR, Johnson WE, O'Brien SJ, Ramos MJ, Antunes A (2008) The adaptive evolution of the mammalian mitochondrial genome. *BMC Genomics*, **9**, 119.
- Fuentes I, Stegemann S, Golczyk H, Karcher D, Bock R (2014) Horizontal genome transfer as an asexual path to the formation of new species. *Nature*, **511**, 232–235.
- Galmes J, Andralojc PJ, Kapralov MV *et al.* (2014) Environmentally driven evolution of Rubisco and improved photosynthesis and growth within the C3 genus *Limonium* (Plumbaginaceae). *New Phytologist*, **203**, 989–999.
- Galtier N, Nabholz B, Glémin S, Hurst GD (2009) Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology*, **18**, 4541–4550.
- Gordon VS, Staub JE (2011) Comparative analysis of chilling response in cucumber through plastidic and nuclear genetic effects component analysis. *Journal of the American Society for Horticultural Science*, **136**, 256–264.
- Greiner S (2012) Plastome mutants of higher plants. In: *Genomics of Chloroplasts and Mitochondria* (eds Bock R, Knoop V), pp. 237–266. Springer, Dordrecht, Heidelberg, New York, London, the Netherlands.
- Greiner S, Bock R (2013) Tuning a ménage à trois: co-evolution and co-adaptation of nuclear and organelle genomes in plants. *BioEssays*, **35**, 354–365.
- Guisinger MM, Kuehl JV, Boore JL, Jansen RK (2008) Genome-wide analyses of Geraniaceae plastid DNA reveal unprecedented patterns of increased nucleotide substitutions. *Proceedings of the National Academy of Sciences USA*, **105**, 18424–18429.
- Haag-Liautard C, Coffey N, Houle D *et al.* (2008) Direct estimation of the mitochondrial DNA mutation rate in *Drosophila melanogaster*. *PLoS Biology*, **6**, e204.
- Hansen AK, Escobar LK, Gilbert LE, Jansen RK (2007) Paternal, maternal, and biparental inheritance of the chloroplast genome in *Passiflora* (Passifloraceae): implications for phylogenetic studies. *American Journal of Botany*, **94**, 42–46.
- Hao DC, Chen SL, Xiao PG (2010) Molecular evolution and positive Darwinian selection of the chloroplast maturase matK. *Journal of Plant Research*, **123**, 241–247.
- Hill WG, Robertson A (1966) The effect of linkage on limits to artificial selection. *Genetical Research*, **8**, 269–294.
- Hill JH, Chen Z, Xu H (2014) Selective propagation of functional mitochondrial DNA during oogenesis restricts the transmission of a deleterious mitochondrial variant. *Nature Genetics*, **46**, 389–392.
- Hough J, Hollister JD, Wang W, Barrett SCH, Wright SI (2014) Genetic degeneration of old and young Y chromosomes in the flowering plant *Rumex hastatulus*. *Proceedings of the National Academy of Sciences USA*, **111**, 7713–7718.
- Iida S, Miyagi A, Aoki S *et al.* (2009) Molecular adaptation of *rbcl* in the heterophyllous aquatic plant *Potamogeton*. *PLoS One*, **4**, e4633.

- Irwin DE (2012) Local adaptation along smooth ecological gradients causes phylogeographic breaks and phenotypic clustering. *The American Naturalist*, **180**, 35–49.
- Jaramillo-Correa JP, Bousquet J (2005) Mitochondrial genome recombination in the zone of contact between two hybridizing conifers. *Genetics*, **171**, 1951–1962.
- Kapralov MV, Filatov DA (2006) Molecular adaptation during adaptive radiation in the Hawaiian endemic genus *Schiedea*. *PLoS One*, **1**, e8.
- Kapralov MV, Filatov DA (2007) Widespread positive selection in the photosynthetic Rubisco enzyme. *BMC Evolutionary Biology*, **7**, 73.
- Kapralov MV, Kubien DS, Andersson I, Filatov DA (2011) Changes in Rubisco kinetics during the evolution of C4 photosynthesis in *Flaveria* (Asteraceae) are associated with positive selection on genes encoding the enzyme. *Molecular Biology and Evolution*, **28**, 1491–1503.
- Kapralov MV, Smith JAC, Filatov DA (2012) Rubisco evolution in C4 eudicots: an analysis of Amaranthaceae *sensu lato*. *PLoS One*, **7**, e52974.
- Khakhlova O, Bock R (2006) Elimination of deleterious mutations in plastid genomes by gene conversion. *The Plant Journal*, **46**, 85–94.
- Kimball S, Campbell DR, Lessin C (2008) Differential performance of reciprocal hybrids in multiple environments. *Journal of Ecology*, **96**, 1306–1318.
- Kimura M (1983) *The Neutral Theory of Molecular Evolution*. Cambridge University Press, Cambridge.
- Krause K (2012) Plastid genomes of parasitic plants: a trail of reductions and losses. In: *Organelle Genetics* (ed. Bullerwell C). Springer, Berlin.
- Leinonen PH, Remington DL, Savolainen O (2011) Local adaptation, phenotypic differentiation and hybrid fitness in diverged natural populations of *Arabidopsis lyrata*. *Evolution*, **65**, 90–107.
- Leinonen P, Remington DL, Leppala J, Savolainen O (2013) Genetic basis of local adaptation and flowering time variation in *Arabidopsis lyrata*. *Molecular Ecology*, **22**, 709–723.
- Lin Z, Kong H, Nei M, Ma H (2006) Origins and evolution of the *recA/RAD51* gene family: evidence for ancient gene duplication and endosymbiotic gene transfer. *Proceedings of the National Academy of Sciences USA*, **103**, 10328–10333.
- Llopart A, Herrig D, Brud E, Stecklein Z (2014) Sequential adaptive introgression of the mitochondrial genome in *Drosophila yakuba* and *Drosophila santomea*. *Molecular Ecology*, **23**, 1124–1236.
- Maheshwari S, Barbash DA (2011) The genetics of hybrid incompatibilities. *Annual Review of Genetics*, **45**, 331–355.
- Maréchal A, Brisson N (2010) Recombination and the maintenance of plant organelle genome stability. *New Phytologist*, **186**, 299–317.
- Mariac C, Scarcelli N, Pouzadou J *et al.* (2014) Cost-effective enrichment hybridization capture of chloroplast genomes at deep multiplexing levels for population genetics and phylogeography studies. *Molecular Ecology Resources*, **14**, 1109–1113.
- McCaughey DE (2013) Paternal leakage, heteroplasmy, and the evolution of plant mitochondrial genomes. *New Phytologist*, **200**, 966–977.
- McKinnon GE, Vaillancourt RE, Steane DA, Potts BM (2004) The rare silver gum, *Eucalyptus cordata*, is leaving its trace in the organellar gene pool of *Eucalyptus globulus*. *Molecular Ecology*, **13**, 3751–3762.
- Mower JP, Touzet P, Gummow JS, Delph LF, Palmer JD (2007) Extensive variation in synonymous substitution rates in mitochondrial genes of seed plants. *BMC Evolutionary Biology*, **7**, 135.
- Muir G, Filatov D (2007) A selective sweep in the chloroplast DNA of dioecious *Silene* (section *Elisanthe*). *Genetics*, **177**, 1239–1247.
- Nachman MW (1998) Deleterious mutations in animal mitochondrial DNA. *Genetica*, **102–103**, 61–69.
- Neale DB, Wheeler NC, Allard RW (1986) Paternal inheritance of chloroplast DNA in Douglas-fir. *Canadian Journal of Forest Research*, **16**, 1152–1154.
- Neiva J, Pearson G, Valero M, Serrão E (2010) Surfing the wave on a borrowed board: range expansion and spread of introgressed organellar genomes in the seaweed *Fucus ceranoides* L. *Molecular Ecology*, **19**, 4812–4822.
- Nielsen R (2001) Statistical tests of selective neutrality in the age of genomics. *Heredity*, **86**, 641–647.
- Osada N, Akashi H (2012) Mitochondrial–nuclear interactions and accelerated compensatory evolution: evidence from the primate cytochrome c oxidase complex. *Molecular Biology and Evolution*, **29**, 337–346.
- Palme A, Su Q, Palsson S, Lascoux M (2004) Extensive sharing of chloroplast haplotypes among European birches indicates hybridization among *Betula pendula*, *B. pubescens* and *B. nana*. *Molecular Ecology*, **13**, 167–178.
- Parkinson CL, Mower JP, Qiu YL *et al.* (2005) Multiple major increases and decreases in mitochondrial substitution rates in the plant family Geraniaceae. *BMC Evolutionary Biology*, **5**, 73.
- Parks M, Cronn R, Liston A (2009) Increasing phylogenetic resolution at low taxonomic levels using massively parallel sequencing of chloroplast genomes. *BMC Biology*, **7**, 84.
- Petit RJ, Pineau E, Demesure B, Bacilieri R, Ducouso A, Kremer A (1997) Chloroplast DNA footprints of postglacial recolonization by oaks. *Proceedings of the National Academy of Sciences USA*, **94**, 9996–10001.
- Petit RJ, Csaiikl UM, Bordacs S *et al.* (2002a) Chloroplast DNA variation in European white oaks: phylogeography and patterns of diversity based on data from over 2600 populations. *Forest Ecology and Management*, **156**, 5–26.
- Petit RJ, Latouche-Hallé C, Pemonge M-H, Kremer A (2002b) Chloroplast DNA variation of oaks in France and the influence of forest fragmentation on genetic diversity. *Forest Ecology and Management*, **156**, 115–130.
- Petit RJ, Bodénès C, Ducouso A, Roussel G, Kremer A (2004) Hybridization as a mechanism of invasion in oaks. *New Phytologist*, **161**, 151–164.
- Petit RJ, Duminil J, Fineschi S *et al.* (2005) Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. *Molecular Ecology*, **14**, 689–701.
- Powles SB, Yu Q (2010) Evolution in action: plants resistant to herbicides. *Annual Review of Plant Biology*, **61**, 317–347.
- Rand DM, Kann LM (1998) Mutation and selection at silent and replacement sites in the evolution of animal mitochondrial DNA. *Genetica*, **102–103**, 393–407.
- Rieseberg LH, Soltis DE (1991) Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants*, **5**, 65–84.

- Ruiz-Pesini E, Mishmar D, Brandon M, Procaccio V, Wallace DC (2004) Effects of purifying and adaptive selection on regional variation in human mtDNA. *Science*, **303**, 223–226.
- Sambatti JBM, Ortiz-Barrientos D, Baack EJ, Rieseberg LH (2008) Ecological selection maintains cytonuclear incompatibilities in hybridizing sunflowers. *Ecology Letters*, **11**, 1082–1091.
- Schaal BA, Hayworth DA, Olsen KM, Rauscher JT, Smith WA (1998) Phylogeographic studies in plants: problems and prospects. *Molecular Ecology*, **7**, 465–474.
- Schmitz-Linneweber C, Kushnir S, Babiychuk E *et al.* (2005) Pigment deficiency in nightshade/tobacco cybrids is caused by the failure to edit the plastid ATPase alpha-subunit mRNA. *Plant Cell*, **17**, 1815–1828.
- Scowcroft WR (1979) Nucleotide polymorphism in chloroplast DNA of *Nicotiana debneyi*. *Theoretical and Applied Genetics*, **55**, 133–137.
- Sen L, Fares M, Liang B *et al.* (2011) Molecular evolution of *rbcl* in three gymnosperm families: identifying adaptive and coevolutionary patterns. *Biology Direct*, **6**, 29.
- Sloan DB, Taylor DR (2012) Evolutionary rate variation in organelle genomes: the role of mutational processes. In: *Organelle Genetics: Evolution of Organelle Genomes and Gene Expression* (ed. Bullerwell CE), pp. 123–146. Springer-Verlag, Berlin.
- Sloan DB, Alverson AJ, Wu M, Palmer JD, Taylor DR (2012) Recent acceleration of plastid sequence and structural evolution coincides with extreme mitochondrial divergence in the angiosperm genus *Silene*. *Genome Biology and Evolution*, **4**, 294–306.
- Sloan DB, Triant DA, Forrester NJ *et al.* (2014a) A recurring syndrome of accelerated plastid genome evolution in the angiosperm tribe *Sileneae* (Caryophyllaceae). *Molecular Phylogenetics and Evolution*, **72**, 82–89.
- Sloan DB, Triant DA, Wu M, Taylor DR (2014b) Cytonuclear interactions and relaxed selection accelerate sequence evolution in organelle ribosomes. *Molecular Biology and Evolution*, **31**, 673–682.
- Stegemann S, Keuthe M, Greiner S, Bock R (2012) Horizontal transfer of chloroplast genomes between plant species. *Proceedings of the National Academy of Sciences USA*, **109**, 2434–2438.
- Stewart JB, Freyer C, Elson JL *et al.* (2008) Strong purifying selection in transmission of mammalian mitochondrial DNA. *PLoS Biology*, **6**, e10.
- Straub SC, Parks M, Weitemier K, Fishbein M, Cronn RC, Liston A (2012) Navigating the tip of the genomic iceberg: next generation sequencing for plant systematics. *American Journal of Botany*, **99**, 349–364.
- Timmis JN, Ayliffe MA, Huang CY, Martin W (2004) Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. *Nature Reviews Genetics*, **5**, 123–135.
- Toews DPL, Brelsford A (2012) The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*, **21**, 3907–3930.
- Toews DPL, Mandic M, Richards JG, Irwin DE (2013) Migration, mitochondria and the yellow-rumped warbler. *Evolution*, **68**, 241–255.
- Tsitroni A, Kirkpatrick M, Levin DA (2003) A model for chloroplast capture. *Evolution*, **57**, 1776–1782.
- Wallace DC (2005) A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annual Review of Genetics*, **39**, 359–407.
- Weng ML, Ruhlman TA, Gibby M, Jansen RK (2012) Phylogeny, rate variation, and genome size evolution in *Pelargonium* (Geraniaceae). *Molecular Phylogenetics and Evolution*, **64**, 654–670.
- Weng ML, Blazier JC, Govindu M, Jansen RK (2014) Reconstruction of the ancestral plastid genome in Geraniaceae reveals a correlation between genome rearrangements, repeats, and nucleotide substitution rates. *Molecular Biology and Evolution*, **31**, 645–659.
- Wolfe KH, Li W-H, Sharp PM (1987) Rates of nucleotide substitutions vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proceedings of the National Academy of Sciences USA*, **84**, 9054–9058.
- Wright SI, Nano N, Foxe JP, Dar VN (2008) Effective population size and tests of neutrality at cytoplasmic genes in *Arabidopsis*. *Genetics Research*, **90**, 119–128.
- Zhong B, Yonezawa T, Zhong Y, Hasegawa M (2009) Episodic evolution and adaptation of chloroplast genomes in ancestral grasses. *PLoS One*, **4**, e5297.
- Zhu A, Guo W, Jain K, Mower JP (2014) Unprecedented heterogeneity in the synonymous substitution rate within a plant genome. *Molecular Biology and Evolution*, **31**, 1228–1236.

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Glossary

Chondriome: mitochondrial genome.

dN/dS ratio: the ratio of nonsynonymous changes per nonsynonymous site to synonymous changes per synonymous site. When $dN/dS > 1$ positive selection is inferred; when $dN/dS = 1$ neutrality is inferred; and when $dN/dS < 1$ purifying selection is inferred.

Gene conversion: the process by which sequence information from one allele is transferred unidirectionally to an allele of the same locus, or to an allele at a different locus, via mismatch repair in regions of DNA heteroduplex during recombination.

Neutral theory of molecular evolution: a hypothesis that maintains that evolution of DNA sequences occurs primarily by neutral genetic drift rather than natural selection.

Plasmotype: the sum of genetic information contained in the mitochondrial and chloroplast genomes.

Plastome: plastid or chloroplast genome.

Positive selection: natural selection that increases the frequency of beneficial mutations.

Purifying selection: natural selection that removes deleterious mutations.

Selective sweep: rapid increase in the frequency of a genomic region (an allele and neighbouring genomic regions in tight linkage to it) under positive selection.