and fungi (*20*) is a derived character indicating that the root cannot be within opisthokonts. If it is outside bikonts and opisthokonts, it must be at or near the bifurcation between them. Our inability to amplify fusion genes in Amoebozoa (*Phreatamoeba*, *Phalansterium*) does not prove their absence. We also searched the genomic/EST databases of other Amoebozoa (*Dictyostelium*, *Entamoeba histolytica*) for the fusion and individual genes, without success. This is not surprising, for *E. histolytica* and *invadens* lack DHFR or TS enzymatic activity (*21*) and presumably also the genes, whereas *Dictyostelium* probably replaced TS by a nonhomologous enzyme (*22*). If other Amoebozoa have the fusion gene, contrary to present indications, they must be sisters to bikonts and the tree is rooted precisely as in Fig. 1, i.e., between opisthokonts and Amoebozoa/bikonts. If they genuinely lack it, their position will remain ambiguous; they could be sisters of bikonts or opisthokonts or branch below either.

Three arguments suggest, albeit indecisively, that the root may be between opisthokonts and Amoebozoa/bikonts (*5*). First, opisthokonts typically have flat mitochondrial cristae, whereas Amoebozoa/bikonts would ancestrally have had tubular cristae; this difference could reflect divergent specialization immediately following the symbiogenetic origin of mitochondria (*23*). Second, the single cilium is posterior in opisthokonts, but anterior in Amoebozoa; the latter character is shared with bikonts, ancestrally with one anterior and one posterior cilium (*5*). Third, bootstrap support for the bipartition between opisthokonts and bikonts/Amoebozoa is typically much stronger on single-gene trees than that between Amoebozoa and other eukaryotes (*2*, *5*, *7*, *20*); a significantly earlier divergence between opisthokonts and Amoebozoa/bikonts would simply explain this (*5*). Only if Amoebozoa turn out to branch below the opisthokont/bikont bifurcation would they be early diverging eukaryotes—the only ones. If Amoebozoa are sisters of bikonts or opisthokonts, there would be no extant eukaryote lineages that diverged before the common ancestor of animals and plants; the recent extensive searches for early diverging eukaryotes would have been wild goose chases. Further study of genetic diversity within Amoebozoa should clarify their position and thereby precisely pinpoint the root.

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Supporting Online Material

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Materials and Methods

Table S1

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Rapid Regulation of Light Harvesting and Plant Fitness in the Field

Carsten Külheim,[1] Jon Ågren,[2] Stefan Jansson[1]*

We used *Arabidopsis thaliana* mutants to examine how a photosynthetic regulatory process, the qE-type or ΔpH-dependent nonphotochemical quenching, hereafter named feedback de-excitation, influences plant fitness in different light environments. We show that the feedback de-excitation is important for plant fitness in the field and in fluctuating light in a controlled environment but that it does not affect plant performance under constant light conditions. Our findings demonstrate that the feedback de-excitation confers a strong fitness advantage under field conditions and suggest that this advantage is due to the increase in plant tolerance to variation in light intensity rather than tolerance to high-intensity light itself.

The ability to adjust metabolic processes to a variable environment should be crucial for the Darwinian fitness of plants and other sessile organisms, which cannot move away from unfavorable conditions. In recent years, the molecular basis of various short-term regulatory processes has been identified in plants, but the adaptive importance of these processes has never been explored under field conditions. One metabolic pathway that must be strictly controlled is the photosynthetic light reaction because it has potentially dangerous side effects. If the incident light increases or the photosynthetic dark reactions are retarded (for example, due to a drop in temperature or closure of stomata), then there is the risk that the production of adenoside triphosphate (ATP) and the reduced form of nicotinamide adenine dinucleotide (NADPH) by the photosynthetic light reactions becomes greater than the capacity to catabolize these compounds, which causes photo-oxidative stress. Plants have evolved several protective mechanisms that have been suggested to represent adaptations against photo-oxidative stress (*1*). They operate at different time scales, and one, the qE-type of nonphotochemical quenching (NPQ) or feedback de-excitation, is a very rapid process that is induced seconds after a plant has been exposed to extreme light ("high light"). Feedback de-excitation accounts for about 80% of NPQ (*2*) and works by switching the photosynthetic antenna into a state of thermal dissipation instead of efficient solar energy utilization (*3*). Two proteins have been shown to be essential for feedback de-excitation. One is the enzyme violaxanthin de-epoxidase (VDE), which converts one carotenoid species (violaxanthin) to another (zeaxanthin) in the so-called xanthophyll cycle (*4*). The other is the PsbS protein that undergoes a conformational change when the "excitation pressure" rises, resulting in a nonradiative energy dis-

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sipation through feedback de-excitation (*5*).

Two *Arabidopsis thaliana* mutants (*npq1* and *npq4*) that lack the essential proteins for feedback de-excitation have been characterized (*2, 6*). The *npq4* mutant lacks the PsbS protein, whereas the *npq1* mutant lacks VDE and, as a result, both mutants lack the feedback de-excitation. The *npq1* mutant also lacks another of the photoprotective functions associated with carotenoids, the protection against high light–induced damage to membrane lipids associated with zeaxanthin formation (*7*). Short-term exposure of *npq1* to a combination of high light and cold temperature leads to transient photodamage (*8*). Surprisingly, both mutants grow, however, as do wild-type plants in the laboratory even under high light conditions (*6*). These results, also observed in our own laboratory, raise doubts about the adaptive significance of feedback de-excitation for plant performance. We speculated that the feedback de-excitation is maintained by selection because it provides tolerance to rapidly fluctuating excitation pressure rather than protection against high light conditions. To test this hypothesis, we compare here the performance of *npq1* and *npq4* mutants with that of the corresponding wild type (Columbia) when grown under field conditions in an experimental garden and when grown in a climate chamber under light of constant or variable intensity. By including both the *npq1* and the *npq4* mutants, we were able to evaluate the relative importance of the feedback de-excitation and the protection against lipid damage associated with zeaxanthin production under different light environments.

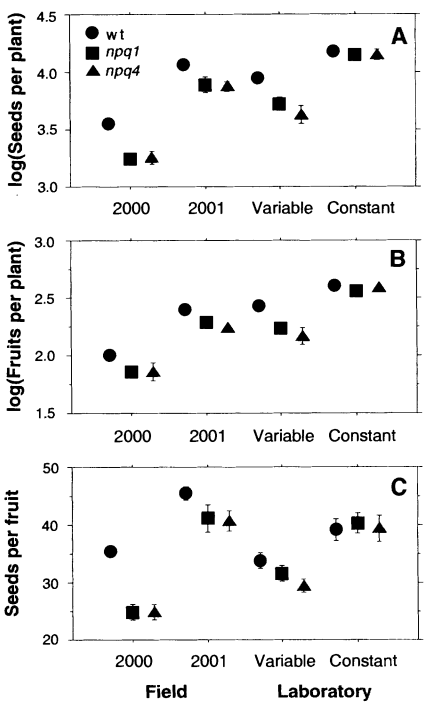

Fig. 1. Reduced fitness of *Arabidopsis* mutants. (**A**) Log (number of seeds per plant); (**B**) log (number of fruits per plant); (**C**) number of seeds per fruit. Plants representing three genotypes (*npq1*, *npq4*, and wild type) were grown (i) in a randomized block design for 2 years in the experimental garden of Umeå University and (ii) in the climate chamber under variable and constant light regimes (23° to 18°C, humidity 80%, and light intensity 90 to 270 in a 30-s period or 180 μmol photons $m^{-2}s^{-1}$, respectively).

Arabidopsis thaliana is an annual plant that flowers early in the season in temperate regions. The seeds germinate either in the same season or in the following spring. Thus, provided that germination and seedling establishment do not vary, lifetime seed production provides an accurate measure of net fitness.

In a first experiment, we grew the plants outdoors in a garden at Umeå, Northern Sweden, in two consecutive years (*9*). To provide the plants with conditions resembling as closely as possible those of a natural population, they were not watered, fertilized, or treated with pesticides. The plants were simultaneously exposed to several kinds of environmental stress. In comparison to plants grown in the climate chamber, the plants grown in the garden flowered at a smaller size, produced smaller and thicker leaves, showed stress symptoms (anthocyanin accumulation), and were subject to some grazing by snails and arthropods. Under field conditions, the two mutant genotypes produced about 50% fewer seeds than the wild type in 2000 and about 30% fewer seeds in 2001 (Fig. 1A, Table 1). The difference in seed output occurred because (i) the mutants produced about 25% fewer fruits than the wild type in both years (Fig. 1B) and (ii) they produced about 28 and 9% fewer seeds per fruit compared with the wild type in 2000 and 2001, respectively (Fig. 1C, Table 1). The three genotypes did not differ significantly in flowering time or seed weight (data not shown). Thus, the fitness of plants lacking feedback de-excitation was greatly reduced under field conditions.

The feedback de-excitation is thought to protect photosystem II (PS II) from photoinhibition, i.e., slowly reversible reduction in quantum efficiency of electron transport of PS II due to photo-oxidative damage or sustained thermal dissipation, which is caused by excess light (*10*). Photoinhibition is most conveniently quantified as the decrease in the chlorophyll fluorescence parameter F_v/F_m (*9*) of intact plants. To determine the extent to which the plants in the outdoor experiment experienced photoinhibition, we measured F_v/F_m of the experimental plants in the garden. The level of photoinhibition varied from day to day but tended to increase with age. On cloudy days, neither the wild type nor the two mutants experienced photoinhibition. However, the higher the photoinhibition (and, thus, the stronger the reduction in F_v/F_m) in wild-type plants, the larger the difference between the genotypes (Fig. 2). Photoinhibition follows a diurnal pattern, normally peaking around noon (termed "midday repression") when the light intensity is at its maximum. Midday repression was more pronounced for the mutants than for the wild-type plants (data not shown). These results confirm that the feedback de-excitation protected against photoinhibition.

The photosynthetic apparatus was subject to photodamage in the field, but is high light itself stressful to the mutants? The photosynthetic apparatus adjusts in several ways to high light, for example, by increasing the amount of Calvin cycle enzymes and decreasing the size of the photosynthetic antenna. If these long-term adjustments are sufficient, the feedback de-excitation will not go into operation. However, rapid fluctuations in excitation pressure must be handled by short-term regulatory mechanisms like the feedback de-excitation. At the experimental site, we recorded the amount of photosynthetically active radiation (PAR). On a representative day, PAR fluctuated irregularly, typically between 500 and 2000 μmol photons m^{-2} s^{-1}, due to cloudiness and occasional shading by adjacent vegetation (Fig. 3).

To examine whether such short-term variation in excitation pressure rather than the high light could explain the low relative fitness of the mutants under field conditions, we grew the plants in a climate chamber under standard laboratory conditions and under rapidly fluctuating light (*9*). In the latter treatment, the light inten-

Table 1. Reduced fitness of *Arabidopsis* mutants. Two-way ANOVA of the effects on fruit and seed production of *Arabidopsis* of genotype (*npq1*, *npq4*, and wild type) and year (2000 versus 2001) in the field experiment and genotype and light environment (constant versus variable) in the climate chamber.

Source of variation	df	Log (no. fruits per plant)		No. seeds per fruit		Log (no. seeds per plant)	
		MS	F	MS	F	MS	F
		Field experiment					
Genotype	2	0.0463	5.3*	146.8	15.7‡	0.143	15.6‡
Year	1	0.9303	105.8‡	1197.6	128.3‡	2.107	230.2‡
Genotype × year	2	0.0022	0.3	22.5	2.4	0.009	1.0
		Climate chamber					
Genotype	2	0.234	6.2†	49.7	0.6	0.329	6.4†
Light environment	1	3.062	80.8‡	2112.6	25.5‡	5.025	98.2‡
Genotype × light	2	0.159	4.2*	63.2	0.8	0.241	4.7*

*$P < 0.05$. †$P < 0.01$. ‡$P < 0.001$.

sity was varied threefold in a 30-s cycle, and the average light intensity (180 μmol photons $m^{-2}s^{-1}$) and other environmental variables were kept the same as in the control treatment (Fig. 3). The relative fruit and seed production of the three genotypes varied significantly among treatments, as indicated by genotype and light environment interactions (Table 1). There were no significant differences in fruit [one-way analysis of variance (ANOVA), $F_{2,50} = 0.5$, $P = 0.59$] or seed number per plant ($F_{2,50} = 0.1$, $P = 0.89$) among the three genotypes under constant light conditions (Fig. 1). In contrast, under variable light, the *npq1* and *npq4* mutants produced about 35% fewer fruits ($F_{2,50} = 5.4$, $P = 0.008$) and seeds per plant ($F_{2,50} = 8.1$, $P = 0.0009$) than the wild type (Fig. 1). Under variable light conditions, the differences in fitness between the three genotypes mimicked those observed under field conditions.

Our results demonstrate that the feedback de-excitation has a strong effect on plant fitness under field conditions and under variable light conditions in the climate chamber. These results, together with the previous observation that the *npq1* and *npq4* mutants grow as well as the wild-type plants under conditions of constant high light (*6*), suggest that the feedback de-excitation confers an adaptive advantage because it provides short-term photosynthetic regulation rather than a protective mechanism against high light. Rapid and irregular variations in excitation pressure in the field probably result in greater damage to the photosynthetic apparatus in the mutants, which are unable to quickly adjust light harvesting. The reduction in fitness could be caused by decreased photosynthesis and/or indirect effects inside or outside the chloroplast. The lifetime seed production of the *npq1* genotype, which lacks both the feedback de-excitation and the high light–induced zeaxanthin formation, was not lower than that of the *npq4* mutant, which lacks feedback de-excitation only. This indicates that zeaxanthin-dependent lipid protection is less important than feedback de-excitation under the light conditions of the experimental site in northern Sweden.

The contribution to fitness of genes affecting plant development has been investigated (*11*), but this is, to the best of our knowledge, the first study in which the adaptive significance of a mechanism regulating plant primary metabolism has been quantified under field conditions. It shows that experiments under controlled conditions, which are appropriate for dissecting physiological processes, may greatly underestimate the importance of these processes in natural environments. Furthermore, it illustrates the value of *Arabidopsis* as a model system to study the consequences of well-defined genetic differences in the field (*12*). The *Arabidopsis* genome has been completely sequenced (*13*), and mutant genotypes are being isolated at an increasing rate. Within an evolutionary framework, this system offers many opportunities to bridge the gap between molecular biology, physiology, and ecology.

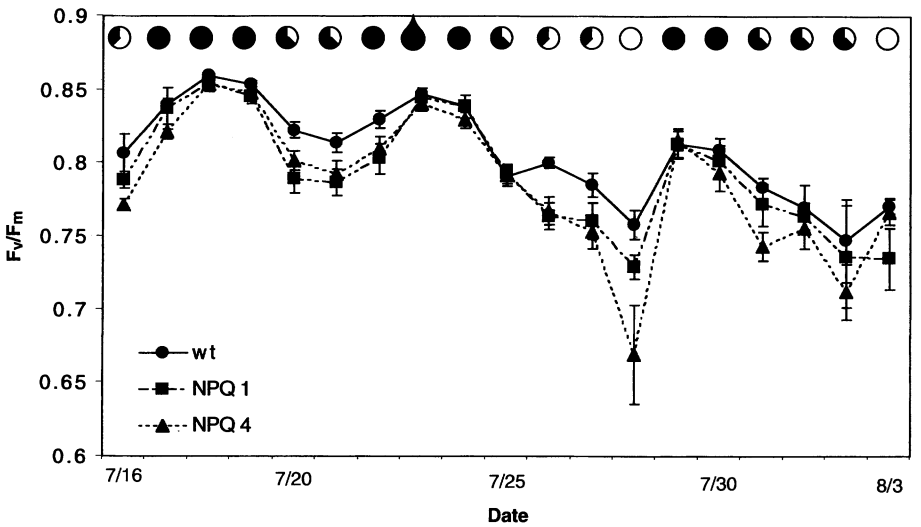

Fig. 2. Photoinhibition in the field. F_v/F_m was measured on intact plants representing the three *Arabidopsis* genotypes (*npq1*, *npq4*, and wild type), grown in the experimental garden each day at noon. Average weather conditions for each measuring day are indicated with symbols at top for full sun, partially cloudy, cloudy, and rain.

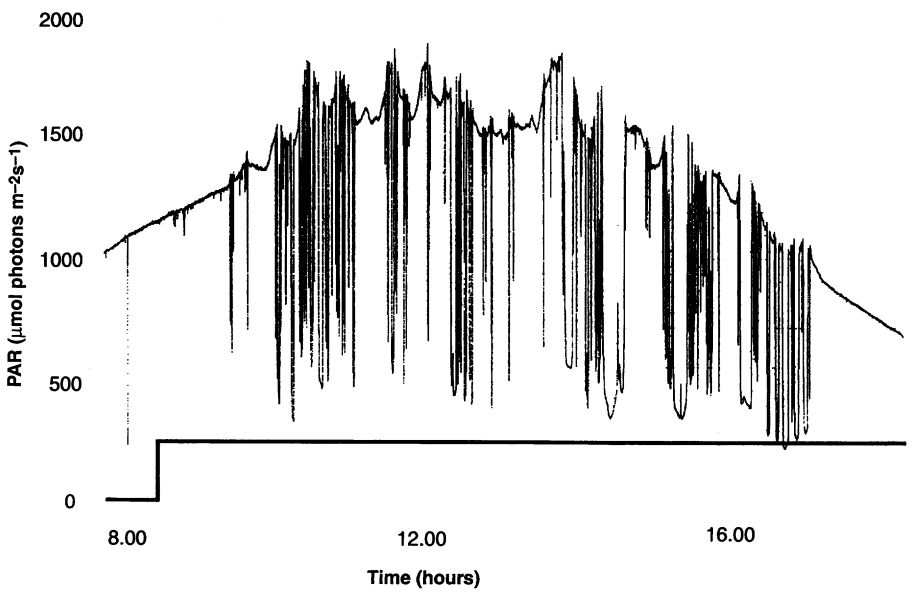

Fig. 3. Light conditions during the experiments. The upper curve shows actual incident light [PAR in μmol photons m^{-2} s^{-1}] measured at the experimental site during one representative day. For comparison, the intensity of the (average) light in the climate rooms is also indicated (solid horizontal line).

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