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Supporting Online Material
www.sciencemag.org/cgi/content/full/315/5817/1416/DC1
Materials and Methods
Figs. S1 to S7
Table S1

References and Notes
Movies S1 and S2

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Ecological Speciation in South Atlantic Island Finches

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Examples of sympatric speciation in nature are rare and hotly debated. We describe the parallel speciation of finches on two small islands in the Tristan da Cunha archipelago in the South Atlantic Ocean. *Nesospiza* buntings are a classic example of a simple adaptive radiation, with two species on each island: an abundant small-billed dietary generalist and a scarce large-billed specialist. Their morphological diversity closely matches the available spectrum of seed sizes, and genetic evidence suggests that they evolved independently on each island. Speciation is complete on the smaller island, where there is a single habitat with strongly bimodal seed size abundance, but is incomplete on the larger island, where a greater diversity of habitats has resulted in three lineages. Our study suggests that the buntings have undergone parallel ecological speciation.

During much of the 20th century, speciation among sexually reproducing organisms was assumed to require an allopatric phase, when the incipient species were isolated (*1*–*3*). Over the past decade, models have been developed suggesting that speciation can occur through natural or sexual selection in parapatry or sympatry, with partial or complete overlap between populations (*4*–*6*). Initial segregation is driven by frequency-dependent disruptive selection, in which individual fitness is determined by the composition of the population through competition. This is termed adaptive speciation to stress the importance of biological interactions (*4*), although adaptive processes also may reinforce segregation in allopatrically derived lineages (*7*, *8*). Ecological speciation is a similar process, whereby reproductive isolation results from divergent selection for different environments or niches, but it makes no assumptions about the initial spatial structure of populations (*7*). It also predicts the independent evolution of convergent ecomorphs in similar environments (*7*).

There is much debate about adaptive sympatric speciation (*2*, *5*, *6*, *9*), with recent theoretical studies suggesting that speciation through competitive interactions is either unlikely (*9*) or plausible only under far more restrictive conditions than originally proposed (*5*, *6*). In sexually reproducing organisms, assortative mating is necessary to reduce gene flow between lineages, although the number of loci affecting a trait under selection may also play a role (*6*). In empirical studies it is difficult to exclude the possibility of initial allopatric segregation and subsequent dispersal (*1*). The most plausible examples are found in host-specific insects and freshwater fish (*4*, *10*, *11*). Among birds, the specialization of brood parasitic species on different hosts may lead to sympatric speciation (*12*), but resource specialization is not known to drive speciation, with intraspecific competition being reduced through sexual dimorphism or, more rarely, through trophic polymorphism (*13*, *14*).

Island finches have been especially influential in the development of evolutionary theory (*15*, *16*). Lack's classic study of Darwin's finches (*16*) provided strong support for the allopatric model of speciation. Although recent studies have shown that hybridization and introgression are important in the evolution of Darwin's finches (*17*–*19*) and that competitive interactions reinforce species differences in sympatry (*8*), the initial development of morphological diversity is still considered to have occurred in isolation (*15*). However, the large number of islands and finch species makes it difficult to infer evolutionary

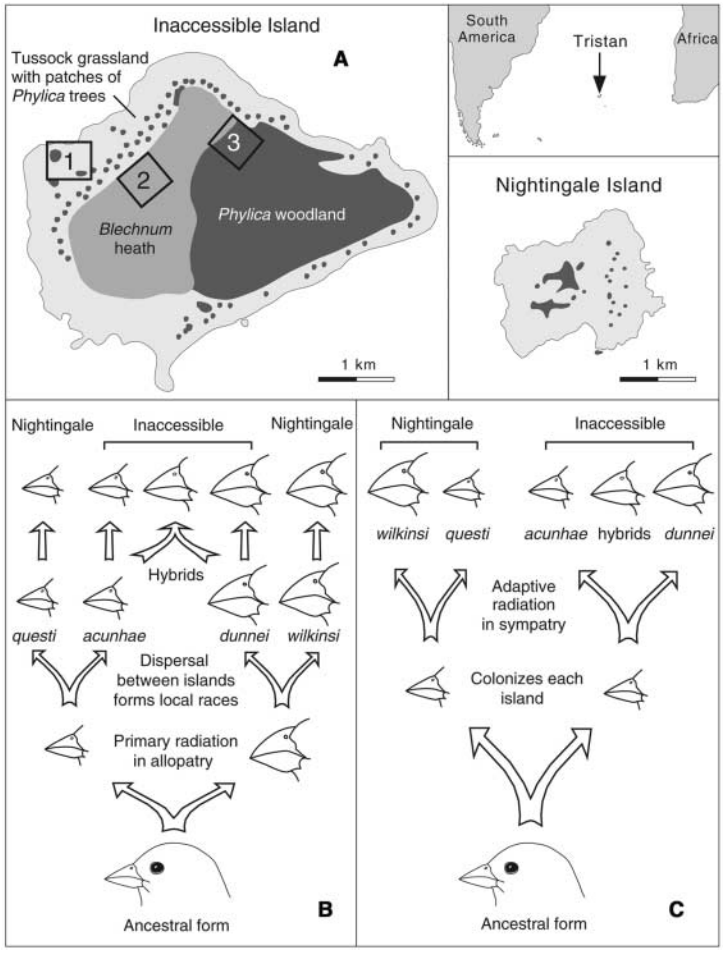

Fig. 1. The Tristan da Cunha archipelago (**A**) showing the distributions of habitat types on Inaccessible Island (14 km^2) and Nightingale Island (4 km^2). Squares 1 to 3 show the main study areas on Inaccessible Island. The diversity of *Nesospiza* buntings could result from either allopatric speciation (**B**) or parallel sympatric radiations (**C**).

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histories (*20*). We provide evidence for parallel ecological speciation in the simpler radiation of *Nesospiza* buntings at Tristan da Cunha (*16*, *21*).

The Tristan da Cunha archipelago comprises three volcanic islands 20 to 35 km apart in the central South Atlantic Ocean (Fig. 1A). The islands differ in size and age, with the largest island (Tristan, 96 km^2) being approximately 200,000 years old, whereas Nightingale Island (4 km^2) is at least 18 million years old (*22*). Inaccessible Island is intermediate in size (14 km^2) and age (3 million years) (*23*). Palynological studies indicate that the vegetation has remained relatively unchanged for at least the past 20,000 years (*23*). Like Darwin's finches (*24*), *Nesospiza* buntings evolved from finch-tanagers (Thraupini). Their South American ancestors were carried on the prevailing westerly winds across 3000 km of ocean (*25*). Vagrant finches also colonized Gough Island, 350 km south of Tristan, giving rise to the endemic Gough bunting *Rowettia goughensis* (*25*). Two *Nesospiza* species are recognized: Tristan bunting *N. acunhae* is an abundant small-billed dietary generalist, whereas Wilkins' bunting *N. wilkinsi* is a scarce large-billed specialist on the woody fruit of *Phylica arborea*. Both species occur on Inaccessible and Nightingale Islands, with separate subspecies on each island (Fig. 1, B and C). *N. acunhae* became extinct on the main island of Tristan about a century ago, after the introduction of mice and rats. A large-billed form was not known to occur on Tristan, despite the presence of *Phylica* trees. The other two islands have been little influenced by humans. We used morphological, ecological, and genetic data to analyze speciation within *Nesospiza* (*25*).

The two species differ greatly in size on Nightingale Island (Fig. 2A), a relatively low-lying island (mostly <300 m) dominated by the tall tussock grass *Spartina arundinacea* and scattered *Phylica* trees (*26*) (Fig. 1A). Inaccessible Island is larger, with an extensive plateau 300 to 600 m above sea level. The coastal lowlands and cliffs support tussock grass and *Phylica* copses, but the plateau has two additional habitats (Fig. 1A). The lower, more sheltered eastern plateau supports *Phylica* woodland, whereas the higher, western plateau lacks *Phylica* trees and is dominated by *Blechnum palmiforme* heath (*26*). This greater diversity of habitat is accompanied by a greater variety of bunting phenotypes (*27*), but their size differences are smaller, with considerable overlap because of hybridization on the eastern plateau (Fig. 2A). Wilkins' (*dunnei*) and Tristan (*acunhae*) buntings co-occur in coastal tussock grass, but *N. w. dunnei* is largely absent from the western plateau, whereas the eastern plateau supports a hybrid swarm of birds with bills ranging between large and small (*27*). In addition, "upland" Tristan buntings on the plateau have distinctly brighter plumage than drably colored "lowland" birds along the coast (*27*).

Bill depth in finches is directly correlated with the crushing force they can exert and thus the size of seeds they can exploit (*15*). Bill size in *Nesospiza* is highly heritable (*28*), with close correspondence between bill depth and seed availability in each habitat (Fig. 2B). Tussock grassland with scattered *Phylica* trees provides a bimodal distribution of seed sizes, favoring the evolution of two finch taxa. Segregation is complete on Nightingale Island. Each species has a distinctive song and defends territories only against conspecifics, and there is no evidence of hybridization (*27*). A similar situation occurs in the same coastal habitat on Inaccessible Island, although one mixed-species pair (<0.1% of all pairs) and occasional hybrids have been observed. On the western plateau, there are no *Phylica* fruit and hence few large-billed birds (Fig. 2B). Sedges predominate, with smaller seeds than *Spartina* tussock grass, favoring the evolution of a smaller-billed bird (upland *acunhae*) than Tristan buntings found at the coast (lowland *acunhae*). The eastern plateau offers relatively low densities of sedge seeds and *Phylica* fruits (Fig. 2B); birds in this area consume more invertebrates than other populations (*29*). Large-billed birds predominate where *Phylica* fruit are abundant, with small-billed birds predominating where there are few fruit, even among adjacent territories (fig. S1). Hybrids have a different song from buntings elsewhere on the island (*29*), but mating is random with respect to phenotype at the ecotone (*25*).

The traditional allopatric model of speciation posits that the two species evolved in isolation, presumably on separate islands, with subsequent dispersal between islands to form local races (Fig. 1B). Alternatively, small- and large-billed forms could have evolved independently on each island (Fig. 1C). This model is supported by sequence data for the mitochondrial gene for cytochrome b (*25*) (fig. S2). There is near-complete lineage sorting by island, with monophyly relative to Gough buntings. Only one Tristan bunting from Nightingale had an allele shared with Inaccessible buntings (*25*), indicating migration or incomplete lineage sorting. This individual was larger than all other Tristan buntings caught on Nightingale, which suggests that it may have arrived from Inaccessible. On the basis of morphology, it was the only case of movement between the islands among 925 birds sampled.

Low levels of cytochrome b allelic diversity (0.536) relative to Darwin's finches (0.933), with comparable levels of nucleotide diversity (*Nesopiza*, 0.023; *Geospiza*, 0.011), are consistent with a small founder population (*25*) and thus provide low resolution. However, microsatellite data also support independent radiations on each island (Table 1 and Fig. 3) (*25*). Bayesian

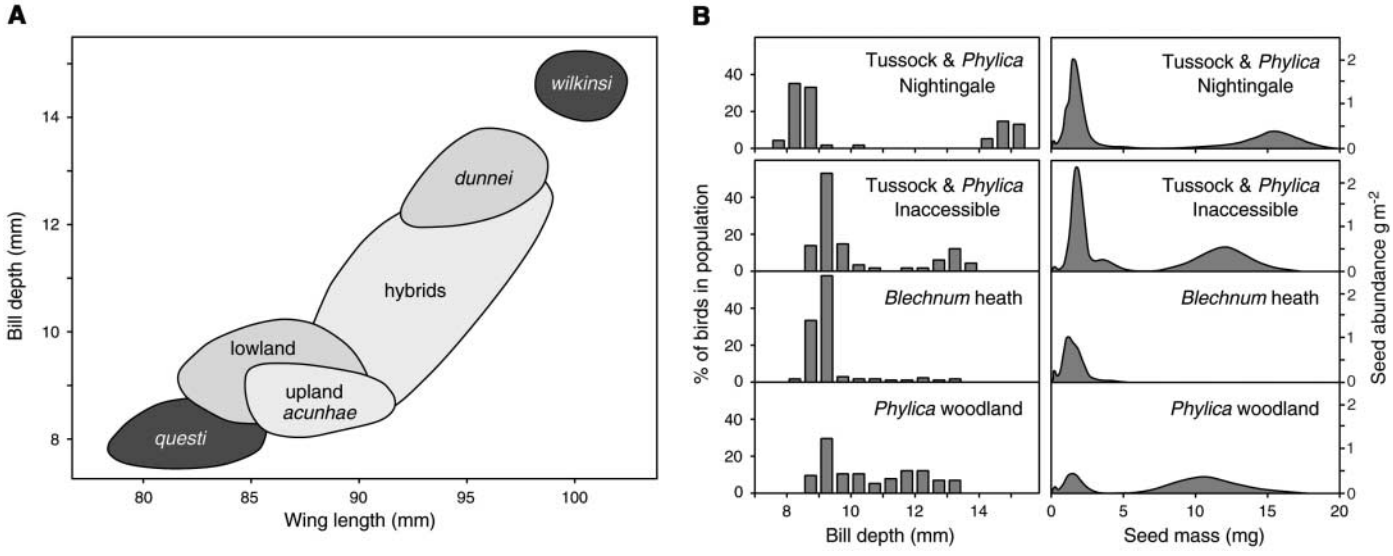

Fig. 2. Variation in male *Nesospiza* bill and body size. (**A**) shows greater morphological segregation on Nightingale Island (*N. w. wilkinsi* and *N. a. questi*) than on Inaccessible Island (all other taxa), where hybridization occurs. Bill sizes closely match peaks in the abundance of seeds of different sizes on Nightingale Island and in each of the three main habitats on Inaccessible Island (**B**).

assignment tests without prior definition of populations identified five lineages when buntings from Tristan and Gough were analyzed together ($n = 4$ loci), discriminating Gough buntings from Tristan (*N. a. questi*) and Wilkins' (*N. w. wilkinsi*) buntings on Nightingale Island (Fig. 3B). Buntings from Inaccessible were distinct from their counterparts on Nightingale Island but initially showed little intraisland structure (Fig. 3B). However, three lineages emerged when they were analyzed separately for seven loci (Fig. 3C). These lineages are not completely sorted, but correspond to *N. w. dunnei* and the two color morphs of *N. a. acunhae*, with hybrids showing an even contribution from the three lineages (Fig. 3C and table S1). The lack of lineage sorting may result either from ongoing speciation or from an equilibrium between selection and recurrent hybridization.

Table 1. Pairwise *F* statistics between morphologically described taxa. All microsatellite (upper matrix, $P < 0.001$) and mtDNA (lower matrix, $P < 0.05$) pairwise *F* statistics indicated significant differentiation. Only nonzero mtDNA *F* statistics are shown. Dashes indicate no difference; blank spaces indicate self-comparisons.

Species	Inaccessible Island				Nightingale Island	
	1	2	3	4	5	6
1. Lowland *N. a. acunhae*		0.053	0.033	0.099	0.262	0.394
2. Upland *N. a. acunhae*	–		0.022	0.087	0.204	0.284
3. Hybrids	–	–		0.033	0.196	0.330
4. *N. w. dunnei*	–	–	–		0.202	0.413
5. *N. a. questi*	0.75	0.52	0.75	0.75		0.425
6. *N. w. wilkinsi*	1.00	0.80	1.00	1.00	–	

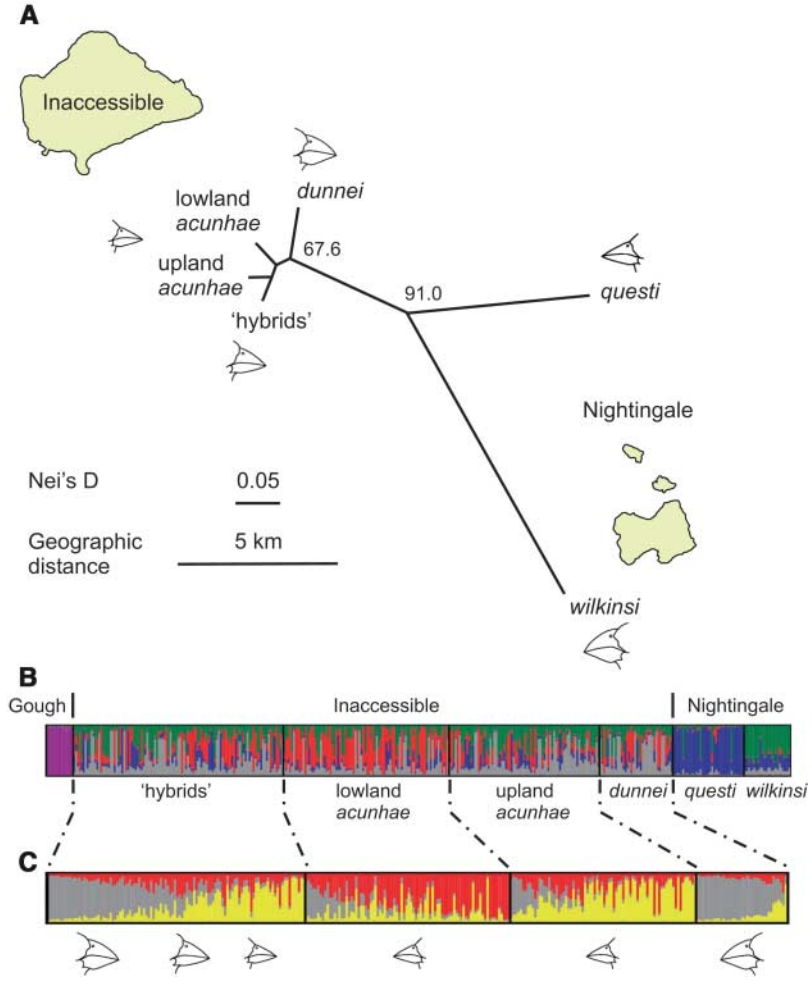

Fig. 3. Phylogeography of *Nesospiza* based on microsatellites. An unrooted dendrogram (**A**) shows clear genetic segregation between island populations (unweighted pair-group method with arithmetic mean based on Nei's unbiased genetic distance *D*). Bayesian assignment probabilities of individuals to lineages, where each vertical line represents an individual and colors indicate the proportion of an individual's genotype assigned to a particular lineage, show clear segregation between islands (**B**), but little structure on Inaccessible when all data are analyzed together (four loci). However, when birds from Inaccessible are analyzed separately (seven loci), there are frequency differences in the assignment of individuals to lineages between morphological taxa (**C**).

Independent radiations on Inaccessible and Nightingale Islands provide a parsimonious explanation for our data (Fig. 1C), especially the origin of three lineages linked to different habitats on Inaccessible Island. We cannot exclude the possibility that initial segregation occurred in allopatry, with genetic similarity between island populations resulting from subsequent hybridization. This would explain why taxa on each island have identical mitochondrial sequences but differ in microsatellites. However, this could result from a founder effect, with the diversity of biparentally inherited microsatellites recovering faster than maternally inherited mitochondrial DNA (mtDNA) because of elevated mutation rates and the reduced impact of a bottleneck on microsatellite allelic diversity. Adaptive sympatric speciation requires frequency-dependent disruptive selection and assortative mating (*4*–*6*). The former is promoted by the high density of buntings (up to 18 pairs ha^{-1} in tussock grassland) (*29*), which favors individuals that exploit novel niches. The latter is promoted by three factors: Juveniles recruit to areas close to their natal area (*29*); plumage color is determined by diet, which varies with habitat (*27*); and vocal differences are linked to phenotype, with small birds having fast, high-pitched songs (*29*). Plumage color and song structure are both important cues in mate selection (*29*). Irrespective of whether segregation occurred initially in sympatry or allopatry, *Nesospiza* provides a compelling example of ecological speciation, because ecological processes appear to be responsible for the evolution and maintenance of morphological diversity, despite localized hybridization on Inaccessible Island.

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Materials and Methods
SOM Text
Figs. S1 and S2
Tables S1 to S4
References

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Coupling Diurnal Cytosolic Ca^{2+} Oscillations to the CAS–IP_3 Pathway in *Arabidopsis*

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Various signaling pathways rely on changes in cytosolic calcium ion concentration ($[Ca^{2+}]_i$). In plants, resting $[Ca^{2+}]_i$ oscillates diurnally. We show that in *Arabidopsis thaliana*, $[Ca^{2+}]_i$ oscillations are synchronized to extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) oscillations largely through the Ca^{2+}-sensing receptor CAS. CAS regulates concentrations of inositol 1,4,5-trisphosphate (IP_3), which in turn directs release of Ca^{2+} from internal stores. The oscillating amplitudes of $[Ca^{2+}]_o$ and $[Ca^{2+}]_i$ are controlled by soil Ca^{2+} concentrations and transpiration rates. The phase and period of oscillations are likely determined by stomatal conductance. Thus, the internal concentration of Ca^{2+} in plant cells is constantly being actively revised.

Organisms, from single-celled to multicellular, exploit the unique physical and chemical properties of the calcium ion to carry out essential biological functions. $[Ca^{2+}]_i$ increases transiently and/or repetitively in response to many abiotic and biotic stimuli (*1*–*3*) and also displays diurnal oscillations in animals and plants (*4*, *5*). In plants, the resting circadian $[Ca^{2+}]_i$ oscillations occur at the whole-tissue level (*6*), in contrast to those seen in specific neurons in animals (*4*, *7*), and are regulated by photoperiod and light intensity (*8*). This oscillating feature implies a robust regulatory machinery that synchronizes $[Ca^{2+}]_i$ throughout the plant. However, due to the lack of the knowledge of sensory receptors and Ca^{2+} channels (*2*, *5*, *9*), the underlying mechanisms for the resting $[Ca^{2+}]_i$ and its oscillations remain largely unknown.

We have cloned a receptor for external Ca^{2+} ($Ca^{2+}{}_o$), CAS, from *Arabidopsis* (*10*). CAS is expressed in the shoot, localizes to the plasma membrane, binds to Ca^{2+}, and mediates $Ca^{2+}{}_o$-induced $[Ca^{2+}]_i$ increases (CICI) in stomatal guard cells. We have generated *CAS* antisense lines (*CASas*) and shown that its mRNA and protein levels are reduced and that CICI is abolished (*10*). Identification of CAS and genetic manipulation of its activity may provide a powerful tool to dissect the mechanisms controlling $[Ca^{2+}]_i$ oscillations. We hypothesized not only that $Ca^{2+}{}_o$ serves as a signal triggering $[Ca^{2+}]_i$ increases, but also that $Ca^{2+}{}_o$ and CAS control the resting $[Ca^{2+}]_i$. To test this hypothesis, we measured the resting $[Ca^{2+}]_i$ using aequorin bioluminescence-based Ca^{2+} imaging (*6*, *8*, *11*) and found that the resting $[Ca^{2+}]_i$ was lower in *CASas* than in wild-type plants (Fig. 1A).

Next, we asked whether $[Ca^{2+}]_i$ oscillations were affected in *CASas*. Biological oscillations can be described by three parameters: amplitude, phase, and period (*12*). Quantitative analysis of leaf aequorin luminescence showed that the amplitudes of $[Ca^{2+}]_i$ oscillations were reduced in *CASas* throughout a long day (Fig. 1B; $P < 0.001$). The average resting $[Ca^{2+}]_i$ and absolute amplitude (peak − trough) were reduced by 46.0 ± 2.3% and 50.2 ± 3.5% ($P < 0.001$), respectively. However, the phase and period were not altered in *CASas* ($P > 0.1$), indicating that CAS is required for maintaining appropriate oscillating amplitudes. Similar results were seen in three *CASas* lines, *CASas1* to *CASas3* (*10*).

The reduced luminescence in *CASas* was not due to low abundance of cytosolic aequorin. Aequorin-expressing *CASas* lines were generated from a cross between *CASas* and a wild-type line carrying the *35S::aequorin* construct. The stability of aequorin expression in wild-type plants was confirmed for eight generations. The *CASas* and wild-type lines had similar aequorin protein levels (fig. S1A), which remained stable throughout a long day (fig. S1B). Finally, the maximum luminescence was identical in both genotypes, as estimated by discharge in excess Ca^{2+}.

Our data prompted us to investigate how $[Ca^{2+}]_o$ is regulated. Ca^{2+} is dissolved in water in the apoplast (extracellular spaces) and transported primarily from the root to the shoot through the transpiration stream (*13*, *14*). The transpiration rate is governed by stomatal conductance, which displays diurnal oscillations (*15*). We reasoned that $[Ca^{2+}]_o$ is synchronized to stomatal-conductance oscillations, and $[Ca^{2+}]_o$ oscillations are perceived by CAS and converted into $[Ca^{2+}]_i$ oscillations. Thus, the soil Ca^{2+}-signaling cascade would be as follows: soil Ca^{2+} → Ca^{2+} uptake and transport → $[Ca^{2+}]_o$ oscillations → CAS → $[Ca^{2+}]_i$ oscillations.

To test this hypothesis, we analyzed whether media Ca^{2+} affects $[Ca^{2+}]_i$. In wild-type plants, $[Ca^{2+}]_i$ was elevated with increases in media Ca^{2+}, whereas in *CASas* this response was reduced (Fig. 1C; $P < 0.001$). We monitored $[Ca^{2+}]_i$ oscillations in plants grown under physiological (1 mM) and elevated (30 mM) Ca^{2+} concentrations. The overall amplitudes of $[Ca^{2+}]_i$ were elevated in both wild-type and *CASas* plants with increases in media Ca^{2+}, but much more so in the wild type, although the phases were not altered (Fig. 1D).

We assessed how media Ca^{2+} regulates $[Ca^{2+}]_o$ and whether $[Ca^{2+}]_o$ oscillates. We monitored $[Ca^{2+}]_o$ directly by expressing aequorin in the apoplast as described previously (*16*). We found that $[Ca^{2+}]_o$ also displayed diurnal oscillations, which were similar in wild-type and *CASas* plants (Fig. 2A). $[Ca^{2+}]_o$ increased with increases in media Ca^{2+}, and both genotypes showed virtually identical responses (Fig. 2B). To ensure equivalent activity of apoplastic aequorin in both genotypes, we measured extracellular aequorin under conditions similar to those for cytosolic aequorin (*17*). The aequorin protein abundance was similar in wild-type and *CASas* plants (fig. S2).

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