REDUCTIONS IN ABSICIC ACID ARE LINKED WITH VIVIPAROUS REPRODUCTION IN MANGROVES

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We investigate physiological mechanisms behind the convergent evolutionary loss of seed dormancy in plant lineages, focusing on mangroves as a model system. More than 60 angiosperm families, including several mangrove taxa, contain species with seeds that are intolerant of drying and do not undergo dormancy. These desiccation-intolerant species occur with disproportionate frequency in wet or coastal tropical habitats. In plants, the hormone abscisic acid (ABA) coordinates both the development of desiccation tolerance during the onset of seed dormancy and whole-organism responses to flooding. Thus, changes in ABA levels and/or modes of action in different plant compartments are implicated in the repeated evolutionary loss of seed dormancy among species of wet habitats. We compare ontogenetic dynamics of ABA levels in embryonic, maternal, and mature vegetative tissue of four phylogenetically independent pairs of related viviparous mangroves and nonviviparous nonmangroves. We demonstrate that ABA levels are consistently lower in embryos of viviparous mangrove taxa than embryos of nonmangrove, nonviviparous sister taxa. In contrast, elevated tissue concentrations of ABA characterize leaves of all mangrove species tested, while ABA levels in maternal tissues vary among mangrove species. These commonalities suggest a functionally important trade-off between the maintenance of embryonic development and the adjustment of the parent tree to salinity stress. This study yields comparative data on seed physiology in naturally occurring desiccation-intolerant species, for which these data are currently scarce, and demonstrates a potentially significant role of phytohormones in the evolution of plant life histories.

Key words: abscisic acid; dormancy; evolution; mangroves; physiology; reproduction; seeds.

The majority of angiosperm seeds undergo a period of metabolic quiescence or “dormancy” that provides an escape from suboptimal germination conditions in seasonal or spatially heterogeneous environments (Baskin and Baskin, 1988; Venable, 1989; Elmqvist and Cox, 1996). In contrast to these “orthodox” seeds (sensu King and Roberts, 1979), over 60 families of predominantly large-seeded tropical species (including a variety of economically important crops and timber) lack dormancy and germinate prior to, or coincident with, abscission from the maternal plant (Roberts, 1973; Corner, 1976; Garwood, 1983; Berjak, Farrant, and Pammenter, 1989; Ng, 1986; van Teichman and van Wyk, 1991, 1994; Elmqvist and Cox, 1996). These seeds rapidly lose viability if they are dried; hence they are termed desiccation intolerant, or “recalcitrant” to storage (King and Roberts, 1979). Seeds of some species, especially mangroves, may germinate viviparously within the fruit and may even tain prodigious seedling sizes while still on the parent tree. While the implications of desiccation intolerance and precocious germination to seed storage, propagation, agriculture, and forestry have long been recognized (Chin and Roberts, 1980), physiological studies only recently have begun to reveal cellular, biochemical, and genetic causes of these phenomena (Vertucci and Farrant, 1995).

Physiologically, “recalcitrant” and viviparous seeds differ from dormant seeds in that the internal seed hydration levels required to sustain metabolism and embryogeny remain comparatively high throughout embryony (MacIntyre, 1987; Farrant, Pammenter, and Berjak, 1988; Berjak, Farrant, and Pammenter, 1989; KerMODE, 1990; Farrant, Pammenter, and Berjak, 1992, 1993; Osborne and Boubriak, 1994; Pammenter et al., 1994). In seeds of dormant species, the development of desiccation tolerance during mid-to-late embryogenesis consistently has been correlated with increases in the hormone abscisic acid (ABA), among other factors (reviewed by Kigel and Galli, 1995). By contrast, data from studies of desiccation-intolerant seeds and hormone mutants suggest that reduced levels and sensitivities to ABA in the embryo may at least in part explain loss of dormancy in these species. Embryos of desiccation-intolerant species, including Theobroma cacao L. (Pence, 1991), Quercus robur L. (Finch-Savage, 1992) and Avicennia marina (Forsk.) Vierh. (Farrant, Pammenter, and Berjak, 1993) exhibit low quantities of ABA throughout ontogeny. Seeds of ABA-deficient and ABA-insensitive mutants of Zea and Arabidopsis exhibit desiccation intolerance, reduced protein accumulation, and lack of dormancy, frequently leading to viviparous germination (Koomneef, Rueling, and Karssen, 1984; Finkelstein and

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Somerville, 1990; McCarty et al., 1991), while mature plants readily wilt (e.g., Neill and Hogan, 1985), proliferate rhizomes (Vartanian, Marcotte, and Giraudat, 1994), and manifest other symptoms of impaired water balance. Application of the ABA inhibitor fluridone at early stages of embryo maturation similarly induces precocious germination in seeds (Fong, Smith, and Koehler, 1983; Oishi and Bewley, 1992). Exogenous addition of ABA to cultured embryos induces and prolongs desiccation tolerance (LePaige-Degivry and Garello, 1991; Meurs et al., 1992), and inhibits germination (reviewed by Rock and Quatrano, 1995). ABA also may be associated with the up-regulation of genes producing stress-related chaperonin proteins, the so-called “dehydrons,” which have been postulated to protect cellular structure during dehydration in tolerant organisms (Close et al., 1993; Farrant et al., 1996; Ingram and Bartels, 1996). Changing osmotic potentials also constitute signals to the drying embryo, and ABA may act independently or coincidentally to transduce these signals (Yamaguchi-Shinozaki and Shinozaki, 1984). These multiple lines of evidence suggest that ABA confers desiccation tolerance on orthodox embryos to prepare them for maturation drying and metabolic quiescence.

Here, we investigate hormonal correlates of viviparous reproduction in multiple mangrove species. “Mangroves,” the 50 or more species of woody plants restricted to protected, saline tropical habitats (sensu Tomlinson, 1986), offer a model system for studying the role of phytohormones in the evolution of seed physiology. Vivipary accompanied by desiccation intolerance appears with unusual frequency in mangroves and coincides with their evolutionarily recent colonization of flooded habitats (Juncosa and Tomlinson, 1988; Fig. 1). Vivipary (sensu stricto) has arisen basally in the tribe Rhizophoreae (Rhizophoraceae), which includes the mangrove genera Rhizophora (eight species), Bruguiera (six spp.), Ceriops (two spp.), and Kandelia (one sp.) (Tomlinson, 1986). Cryptovivipary, in which the embryo grows continuously but does not emerge from the fruit while attached to the maternal plant, has arisen independently in the genera Aegialitis (Plumbaginaceae), Avicennia (Avicenniaceae), Aegiceras (Myrsinaceae), Nypa (Arecaceae), and Pelliciera (Pellicieraceae). These mangrove families are disparate phylogenetically (Chase et al., 1993), and the appearance of vivipary and cryptovivipary constitutes an evolutionary convergence.

We hypothesize that these mangrove embryos may exhibit low levels of ABA relative both to those necessary to maintain osmotic equilibrium in salt-stressed tissues of the maternal tree and to levels found in related, nonviviparous, nonmangrove species. Preliminary support for this hypothesis is available. In the mangrove Avicennia marina, high ABA concentrations (1500 ng/g) in the pericarp accumulate during histodifferentiation, but these levels rapidly drop during reserve deposition, and only very low levels of ABA occur in embryonic tissues. Mature embryos of eight species of mangrove Rhizophoraceae also exhibit low levels of ABA, a feature shared with other desiccation-intolerant species (Farrant et al., 1996). Pannier and Pannier (1975) report reduced levels of the inhibitor B complex in embryos of Rhizophora mangle L. In the only other published study of ABA action on mangrove embryos, Sussex (1975) found that mRNA upregulation in Rhizophora mangle was insensitive to exogenous applications of ABA. Farrant et al. (1993) proposed a general model for desiccation intolerance, in which ABA levels remain low throughout development of recalcitrant seeds. We seek to investigate the generality of this model by extending the comparison to a broad variety of species.

Though data are currently lacking, one might expect that mangroves inhabiting chronically saline environments would exhibit high levels of ABA in adult vegetative tissues. Tissue and xylem concentrations of ABA increase in many plant species subjected to flooding or salinity (e.g., Mansfield and McAnish, 1995). In roots and stomata, as in embryos, ABA regulates cytosolic osmoticum (Robertson et al., 1990; Sharp, Hsiao, and Silk, 1990; Galau, Jakobsen, and Hughes, 1991; Bradford, 1995; Ward, Pei, and Schroeder, 1995). While the localized actions of phytohormones such as ABA are well documented, few studies have attempted to integrate data on the multifaceted ("pleiotropic" sensu Finch and Rose, 1995) effects of hormones in different plant tissues within a single plant (Chapin, Autumn, and Pugnaire, 1993; Voesenek and Blom, 1996). Thus, we compare ABA concentrations among embryonic, maternal (pericarp), and vegetative (leaf) tissues, to document putative compart-
Table 1. Taxonomic identities, localities of collection, and reproductive status of species used in this study. PG = species exhibits precocious germination; NPG = no precocious germination.

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Seed</th>
<th>Locality of collection</th>
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| Rhizophora mangle L. | Rhizophoraceae | PG   | Fairchild Tropical Garden, Miami, Florida; Wee Wee Caye, Belize
| Rhizophora stylosa Griff. | Rhizophoraceae | PG   | Australian Institute of Marine Science, Queensland, Australia |
| Bruguierea gymnorrhiza L. (Lamk.) | Rhizophoraceae | PG   | Durban, South Africa |
| Bruguierea exaristata Ding Hou | Rhizophoraceae | PG   | Australian Institute of Marine Science, Queensland, Australia |
| Ceriops tagal (Perr.) C.B. Robinson | Rhizophoraceae | PG   | Australian Institute of Marine Science, Queensland, Australia |
| Ceriops decandra (Griff.) Ding Hou | Rhizophoraceae | PG   | Australian Institute of Marine Science, Queensland, Australia |
| Kandelia candel (L.) Druce | Rhizophoraceae | PG   | Australian Institute of Marine Science, Queensland, Australia |
| Cassipourea elliptica Sw. (Poir.) | Rhizophoraceae | NPG  | La Selva Biological Station, Costa Rica |
| Aegiceras corniculatum (L.) Blanco | Myrсинacea | PG   | Australian Institute of Marine Science, Queensland, Australia |
| Ardisia eschallonioides L. | Myrсинacea | NPG  | Fairchild Tropical Garden, Miami, Florida |
| Aegialitis annulata R. Brown | Plumbaginaceae | NPG  | Australian Institute of Marine Science, Queensland, Australia |
| Limonium peregrinum L. | Plumbaginaceae | NPG  | Bokbaai, South Africa; Cape Town, South Africa |
| Nypa fruticans (Thunb.) Wurmb. | Areсaceae | NPG  | Madang Lagoon, Papua New Guinea; Matang, peninsular Malaysia |
| Phoenix reclinata Jacq. | Areсaceae | NPG  | Fairchild Tropical Garden, Miami, Florida |

a Samples and voucher photographs collected by E. J. Farnsworth.

b Coll. P. J. Berjak, University of Natal, South Africa.

c Coll. S. Y. Lee, University of Hong Kong.
d Coll. C.-Y. Chiu, Academia Sinica, Taipei, Taiwan.

Embryos of the mangroves Bruguiera gymnorrhiza, Aegiceras corniculatum, Nypa fruticans, and Aegialitis annulata were weighed wet immediately following collection (N = 10 seeds per species, except N = 3 for Nypa), dried at 70°C for 3 days, and weighed to dry to calculate total gravimetric moisture content. Nonviviparous mature embryos of Ardisia eschallonioides, Cassipourea elliptica, Phoenix reclinata, and Limonium peregrinum were treated similarly following their respective collections. In addition, ten whole seeds of each species above (N = 3 for Nypa) were air-dried to ~30% moisture content by mass for 72 h. Embryonic tissues of all species (except Nypa, which was tested only for ABA content; see below) were subsequently tested for viability using the tetrazolium test of Lakon (1949). Embryos of all mangrove species examined here exhibited a mean gravimetric moisture content of 65.9% (~3.8% [SD], N = 30) of wet mass at the late maturation phase, in contrast to the nonviviparous species (mean 40.4% ± 11.4; N = 30, unpaired t test, P < 0.0001). Air-dried nonviviparous seeds were 100% viable after 72 h, while mean viability of the viviparous species tested declined (60% Bruguiera, 30% Aegiceras, and 50% Aegialitis). All viviparous embryos exhibit continual growth from fertilization, throughout embryogenesis, until dispersal, whereas embryo expansion ceases in the viviparous species collected. Dormancy behavior in the field could not be explicitly characterized in the present study. While protracted dormancy sensu stricto may or may not characterize the tropical nonviviparous sister taxa, a period of reduced growth corresponding to metabolic quiescence occurs in these embryos. It is clear that viviparous mangrove embryos diverge from their nonviviparous upland relatives in major aspects of embryo behavior.

At least three seeds of all species (according to availability) were collected at three stages of development (hereafter referred to as stages 1, 2, and 3): (1) the early postfertilization stage corresponding to histodifferentiation in the embryo (<1 mo postpollination); (2) intermediate maturation phase corresponding to maximum embryo size in nonmangrove species and median fruit length in mangroves (1–4 mo postpollination, depending on species); and (3) a late maturation phase just preceding dispersal. The above embryonic phases were identified in the field based on seed size, known phenological patterns of pollination and fruit set, and inspection by microscopic dissection on simultaneously
Fig. 2. Dynamics of ABA concentrations in embryonic tissues through development of mangrove and nonmangroves, in each of four families. Error bars indicate 95% confidence intervals. Nonmangrove species are denoted by dotted lines and open symbols. Mangrove species are denoted by solid symbols. Species pairs examined in each family are listed in Table 1. Data for all mangrove members of the Rhizophoraceae tested are shown: BE = Bruguiera exaristata; BG = B. gymnorhiza; CD = Ceriops decandra; CT = C. tagal; KC = Kandelia candel; RM = Rhizophora mangle; RS = R. stylosa. Developmental stages correspond to: (1) histidifferentiation, (2) mid-maturation, and (3) late maturation (see Materials and Methods for full description).

collected specimens. The mangrove members of the Rhizophoraceae, which exhibit extreme viviparity, were sampled extensively to compare embryonic ABA dynamics among congeneric species and among genera.

For comparisons of maternal vs. embryonic tissues, seeds were separated into maternal (testa and pericarp) and embryonic components; these tissues were assayed separately for ABA content. ABA levels in foliar (nonreproductive) material were also determined on one intact, fully expanded distal leaf collected at the same time and from the same tree from which fruits had been taken (N = 3 per species). Levels of ABA in intact leaves were assessed and compared to mean levels expressed in embryos (pooled over ontogeny).

Radioimmunoassay—Tissues of mangrove and related nonmangrove species were collected during 1996 from Australia, Costa Rica, Belize, Florida, and Taiwan (sources and localities listed in Table 1). Seeds of all species were flash-frozen in liquid nitrogen upon collection or receipt, freeze-dried, and stored at -80°C until use. Leaves of most species (as available) were similarly processed. In addition to assaying the ambient concentrations of ABA in intact tissues, we sought to determine whether viviparous embryos were capable of upregulating ABA production in response to drying. Thus, subsamples of mangrove seeds were set aside and air-dried for 72 h prior to processing. Lyophilized tissues were separated into maternal and embryonic components, ground to a powder in liquid nitrogen, and prepared for radioimmunoassay (RIA), following the methods of Farrant et al. (1993). Three replicate 0.1-g subsamples (representing three different seed samples) of each tissue type were extracted on a shaker for 14 h at 4°C in 2 mL of 70% methanol containing 50 mg/L sodium ascorbate and 20 mg/L butylhydroxy toluene. Samples were centrifuged at 20,000 rpm for 10 min, the supernatant was collected and filtered through a C18 Sep-Pak Column, and the filtrate dried in a Savant vacuum concentrator. Dried pellets were subsequently redissolved in 1 mL of 100% methanol, vortexed and shaken for 30 min, and recentrifuged. Three replicate aliquots of between 100 and 120 μL of the supernatant (depending on optimal quantity determined from serial dilutions for each species, see below) were pipetted into greiner tubes and vacuum-dehydrated. [3H]-ABA (100 μL) and 100 μL of ABA antibody (raised in rabbit asic fluid for the quantitation of free (+)-ABA, provided by the laboratory of E. W. Weiler, methods of Weiler (1980)) were added to each greiner tube, and incubated for 30 min at 37°C with 500 μL bovine serum albumin. The mixture was precipitated with 850 μL 90% ammonium sulphate for 30 min at 25°C for 30 min, pelleted by centrifugation, washed with 1.5 mL 50% ammonium sulphate, repelleted, and the pellet dissolved in 0.25 mL distilled water. Two millilitres of Picofluor scintillation cocktail were added to all tubes; tubes were capped, placed in a Packard Instrument scintillation counter, and counted after 2 h stabilization. Standard curves using nine known concentrations of ABA antigen were performed simultaneously with tissue samples. Corrections for nonspecific binding (using 100 μL [3H]-ABA antigen-tracer and 100 μL H2O instead of antibody) and total tracer activity (determined by counting solutions of 100 μL [3H]-ABA, 250 μL water, and 2 mL cocktail) were incorporated into the standard curve against which the unknown sample ABA concentrations were calculated. Serial dilution series of a parallel set of extracted tissues were also analyzed by RIA to assess interference of secondary compounds in ABA detection and to optimize concentrations of sample with antibodies. Our calculated ABA contents in tissues incorporate these corrections as linear multipliers (methods of Farrant et
Comparisons of ABA levels among maternal and embryonic tissues of viviparous and nonviviparous species—Levels of ABA in maternal tissues of mangroves were generally lower than related nonmangroves at most ontogenetic stages, especially among the Rhizophoraceae and the Arecaceae (Fig. 4). However, these differences were less pronounced than those exhibited by embryos, and the mangrove Myrsinaceae exhibited higher ABA levels in maternal tissue than the nonmangrove Myrsinaceae. Thus, maternal and embryonic ABA levels did not covary in consistent ways among taxa (Fig. 5). In all species, maternal ABA levels were highest during the histodifferentiation stage, and declined thereafter.

Comparisons of ABA levels among reproductive and vegetative tissues in viviparous and nonviviparous species—Among mangrove species, foliar ABA contents were always higher than embryonic ABA contents (Fig. 6). By contrast, foliar ABA contents of nonmangrove embryos were generally lower than embryonic ABA levels, with the exception of the Myrsinaceae in which leaves did not differ significantly from embryonic tissues. Mangroves exhibited consistently higher foliar ABA levels than nonmangroves (Fig. 7), with a significant difference observed in the Rhizophoraceae, Plumbaginaceae, and Arecaceae.

DISCUSSION

We demonstrate that similar modifications in the dynamics of ABA production in developing embryos have occurred in four disparate families of viviparous mangroves. In seeds of the nonviviparous species, ABA reaches an early maximum during development and thereafter declines (Fig. 2), a pattern consistent with many other desiccation-tolerant seeds (Kermode, 1990). In contrast, viviparous mangroves consistently exhibit lower levels of ABA in embryonic tissues throughout embryogenesis relative to their nonviviparous upland sister taxa (Fig. 2). Differences in ABA content between mangroves and nonmangroves are amplified when expressed on a wet-mass basis (data not shown), due to the high water contents of mangrove embryos. These findings accord with other studies showing that reduced ABA levels are associated with recalcitrance in embryos (Kermode, 1990; Pece, 1991; Finch-Savage, 1992; Farrant, Pammenter, and Berjak, 1993; Farrant et al., 1996). The tissue concentrations of ABA reported here are comparable to those reported for both viviparous mutants and desiccation-tolerant seeds (reviewed in Bewley and Black, 1994).

Mangrove embryos have not forfeited the capacity to produce ABA, however. When subjected to drying, mangrove embryos increase production of ABA (Fig. 3). We propose that continually moist conditions inside the developing fruit appear to be conducive to ABA upregulation, and in turn, that ABA concentrations do not attain levels high enough to initiate maturation drying of the embryo. Sussex (1975) suggested that Rhizophora mangle embryos are insensitive to exogenous ABA application. While this study does not investigate the sensitivity of viviparous embryos to ABA, it does indicate that reduced production of ABA may also contribute to
Fig. 4. Dynamics of ABA concentrations in maternal reproductive tissues through development of mangrove and nonmangroves, in each of four families. Error bars indicate 95% confidence intervals. Nonmangrove species are denoted by dotted lines and open symbols. Mangrove species are denoted by solid symbols. Species pairs examined in each family are listed in Table 1; for the Rhizophoraceae, Rhizophora stylosa and Cassipourea elliptica are shown.

Fig. 5. Comparisons between maternal and embryonic ABA concentrations in mangroves (black bars) and nonmangroves (white bars). Paired samples of maternal and embryonic tissues from the same seed were examined; thus paired difference values were obtained and pooled over all developmental stages; bar represents the mean of three mean difference values per species. Positive values indicate that maternal ABA levels exceeded embryonic ABA levels. An asterisk indicates that embryonic and maternal tissue concentrations of ABA differed significantly by nonoverlap of 95% confidence intervals.

Fig. 6. Comparisons between foliar and embryonic ABA concentrations in mangroves (black bars) and nonmangroves (white bars). Paired samples of foliar and embryonic tissues from the same plant were examined; thus paired difference values were obtained and pooled over all developmental stages; bar represents the mean of three mean difference values per species. Positive values indicate that leaf ABA levels exceeded embryonic ABA levels. An asterisk indicates that embryonic and foliar tissue concentrations of ABA differed significantly by nonoverlap of 95% confidence intervals.
the phenomenon of vivipary in the mangrove Rhizophoraceae.

Maternal tissues surrounding the embryo also exhibit low levels of ABA throughout development in some, but not all, of the mangrove species (Fig. 4), indicating that maternal tissues are not universal points of control. Maternal ABA levels in Nypa (Arecaceae) and Rhizophora stylosa (Rhizophoraceae) are significantly lower than those observed in the upland sister taxa, Phoenix and Cassipourea, respectively. However, mangrove and upland taxa of both the Myrsinaceae and Plumbaginaceae share low maternal ABA contents (Fig. 4). Moreover, the cryptoviviparous mangrove Avicennia marina shows high maternal ABA levels, which have been postulated to constrain growth of the precociously germinating embryo (Forrest et al., 1993). Maternal ABA levels in the viviparous mangroves studied here may be insufficient to inhibit embryo expansion, especially in species whose embryonic hypocotyls can physically puncture the pericarp. ABA differentials between embryonic and maternal tissues do not vary consistently among taxa (Fig. 5), suggesting that embryonic ABA production and/or metabolism occurs independently of production in maternal tissues. Such evidence indicates that depressed maternal ABA production per se is not a stable correlate of viviparous reproduction.

Likewise, concentrations of ABA in the endosperm of these species were not assessed in the present study, and these deserve further study. Juncosa (1982) demonstrated that physical expansion of the endosperm may release Rhizophora embryos from maternal inhibition of germination. Endosperm anatomy, its predominance in developing seeds, and its physical role in viviparous germination, all appear to vary greatly among the mangrove taxa surveyed (Tomlinson, 1986), and may not contribute in a consistent way to the physiological evolution of vivipary.

Comparisons between ABA contents in reproductive and vegetative tissues can illuminate relationships between the production of ABA in multiple plant compartments. Mangrove leaves are typically high in ABA content, by comparison to leaves of their upland relatives (Fig. 7). This finding is not unexpected, given that mangroves occupy saline, flooded habitats; ABA production commonly is upregulated in leaves of plants exposed to these conditions (e.g., Mansfield and McAnish, 1995, and references therein). Mean foliar ABA levels exceed mean ABA levels in embryonic tissues of all mangrove species (Fig. 6). A comparable, but opposite partitioning of ABA is noted in nonmangrove species: leaves show lower mean levels of ABA than embryos (Fig. 6). All mangrove taxa show a diminution of ABA from high foliar levels to maternal tissues, indicating that one important shift in ABA regulation may occur at the interfaces of vegetative and reproductive tissues.

Taken together, these data on foliar, maternal, and embryonic compartmentalization of ABA illustrate that while low embryonic tissue concentrations of ABA characterize all four mangrove families, the rough spatial “location” of the evolutionary change in the control point for ABA production may differ among families (Fig. 8). Among the mangroves, levels of ABA drop precipitously between foliar and fruit compartments. The mangrove Rhizophoraceae show higher ABA concentrations in maternal tissues relative to embryonic tissues, indicating a further shift in ABA regulation at the maternal/embryo interface. The nonmangrove members of the Rhizophoraceae and the Arecaceae show a large difference in ABA between foliar and fruit compartments, while levels of ABA vary in a more complex way among compartments in the nonmangrove members of the Myrsinaceae and Plumbaginaceae.

We can only speculate about the possible functional significance of this compartmentalization of ABA in vegetative, maternal, and embryonic tissues of viviparous mangroves. While mangrove leaves may experience chronic desiccation stress imposed by salinity and extreme evaporative demand (Ball, 1996), adaptations of
hormonal physiology, maternal transfer tissues (Wise and Juncosa, 1989), compatible solute production (Farnsworth, 1997), water transport to the embryo, or ion regulation (Joshi, Jamale, and Bhosale, 1975) may in effect "shield" mangrove embryos from osmotic stress and permit embryo growth in normally inhibitory conditions. The precise mechanisms by which embryos are protected from desiccation stress may vary among plant families, but their common consequences in some mangrove species may include depressed ABA production in embryonic tissues and concomitant vivipary. The generality of this hypothesis for all desiccation-intolerant taxa should be tested.

Not all mangroves are viviparous, and thus these apparent hormonal modifications are not prerequisite to evolutionary acquisition of the mangrove habit. Mature embryos of the nonviviparous species Sonneratia alba J. Smith, for example, exhibit high ABA levels comparable to those of upland species (mean 463.7 ± 85.3 ng/g; N = 3 mature embryos). Vivipary, precocious germination, and recalcitrance occur with disproportionately high frequency among taxa of tropical mangrove and wetland habitats (87% of 202 identified species of this seed type originate in tropical wet forest, riverine, flooded, or coastal environments [Farnsworth, 1997]), and reduced ABA content characterizes those species exhibiting these traits that have hitherto been examined (Farrant et al., 1996).

Salient adaptive values of vivipary in mangroves have been well articulated. Early germination may facilitate rooting (MacNae, 1968), buoyancy during sea dispersal (Rabinowitz, 1978), transfer of maternal nutrients to the seedling (Pannier and Pannier, 1975), maintenance of embryonic osmotic equilibrium (Joshi, 1933; Joshi, Jamale, and Bhosale, 1975), and establishment in coarse-grained environments (Elmqvist and Cox, 1996). However, maternal costs associated with vivipary are also potentially significant: numerous attached seedlings may constitute a substantial carbon sink to the maternal plant (Pannier, 1962; Lin and Stemberg, 1995) and a concentrated, apparent resource for herbivores (Farnsworth and Ellison, 1997). While post hoc evidence describes why vivipary may enhance seedling success in marine habitats, and thus why such traits may be fixed selectively in evolution, it does not reveal mechanisms by which vivipary has arisen coincidentally in so many unrelated mangrove taxa (and conversely why such a seemingly "advantageous" strategy has not proliferated among herbaceous, temperate halophytes and other angiosperms). The present study constitutes the first comparative study of multiple independent origins of vivipary examined within an explicitly phylogenetic context. Such a sampling strategy can begin to address hypotheses about how physiological changes occurred during evolution of this trait and provides evidence that regulation of ABA production has been critical during the evolutionary transition from dormant to non-dormant embryos. Because phytohormones critically control multiple aspects of plant life histories, it is reasonable to conjecture that relatively simple evolutionary changes in hormonal control (possibly resulting from altered maternal modulation of environmental stress) may alter many plant behaviors simultaneously (Chapin, Autumn, and Pugnaire, 1993). We propose that studies of shared physiological traits can augment our understanding of the multiple evolutionary origins of complex characters in many taxa.

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