Evolutionary Novelty Is Concentrated at the Edge of Coral Species Distributions

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Evolutionary Novelty Is Concentrated at the Edge of Coral Species Distributions

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Conservation priorities are calculated on the basis of species richness, endemism, and threats. However, areas ranked highly for these factors may not represent regions of maximal evolutionary potential. The relationship between geography and evolutionary innovation was analyzed in a dominant complex of Caribbean reef corals, in which morphological and genetic data concur on species differences. Based on geometric morphometrics of Pleistocene corals and genetically characterized modern colonies, we found that morphological disparity varies from the center to the edge of the Caribbean, and we show that lineages are static at well-connected central locations but split or fuse in edge zones where gene flow is limited. Thus, conservation efforts in corals should focus not only on the centers of diversity but also on peripheral areas of species ranges and population connectivity.

Coral reefs are the most diverse of all marine ecosystems and are increasingly threatened by climate change (*1*, *2*), ocean acidification (*3*), and local anthropogenetic disturbance (*4*). Their structural framework is formed by scleractinian corals, 32.8% of which have been recently categorized as having an elevated risk of extinction (*5*). Current conservation priorities have been established using various approaches, such as biodiversity hotspots, ecoregions, wilderness areas, and megadiversity countries, that focus on areas that are high in biodiversity or endemism or are severely threatened (*6*–*8*). These priority-setting approaches assume that areas of high biodiversity have high levels of endemism, and they target areas that are under the most threat. However, managing reefs on the basis of these approaches alone has been questioned, in part because centers of species richness and endemicity do not coincide in reef corals (*9*, *10*). Moreover, these approaches do not incorporate evolutionary processes. Taxon richness and measures of phylogenetic diversity have been found to be decoupled in terrestrial floras, pointing in general toward the need for a more evolutionary process–based approach to conservation (*7*). Because the geography of evolutionary innovation in reef corals is unknown, we used data from fossil and extant reef corals to examine the distribution of evolutionary innovation across the biodiversity hotspot in the west-central Caribbean (*6*) relative to the edge of species distributions in the eastern Caribbean.

One evolutionary response that has been observed at the geographic margins of many different plant and animal species is introgressive hybridization, an increasingly recognized source of evolutionary innovation and adaptive radiation (*11*, *12*). In reef corals, hybridization has been found at the periphery of species ranges in both the Caribbean and Indo-Pacific regions (*13*–*15*). Although rare on ecological time scales, hybridization has thus been hypothesized to play an important role in reef corals in range expansion and adaptation to changing environments on geological time scales (*15*). Much of the evidence for this hypothesis is from genetic and reproductive data on living corals, which are limited to ecological time scales. Our investigation expands this research to geological time scales.

Under the assumption that genetic and morphological data are correlated, morphological data have been used successfully to distinguish genetically distinct morphospecies and trace patterns in lineages through geological time in marine invertebrates (*16*). We studied the *Montastraea annularis* coral species complex, because the correlation between genetic and morphological data is strongly supported (fig. S1), and hybridization has been recognized in the geological past by studying morphological intermediates between species (*17*). The *M. annularis* complex has been ecologically dominant on Caribbean reefs for >2 million years (*18*, *19*) and has a fossil record extending back >6 million years (*18*). Its geographic distribution is currently restricted to the Caribbean, Gulf of Mexico, and western Atlantic (Florida, the Bahamas, and Bermuda) (*20*) and, unlike that of many terrestrial organisms (*21*), has not changed throughout its history. Today the complex consists of three species: *M. annularis* s.s., *M. faveolata*,

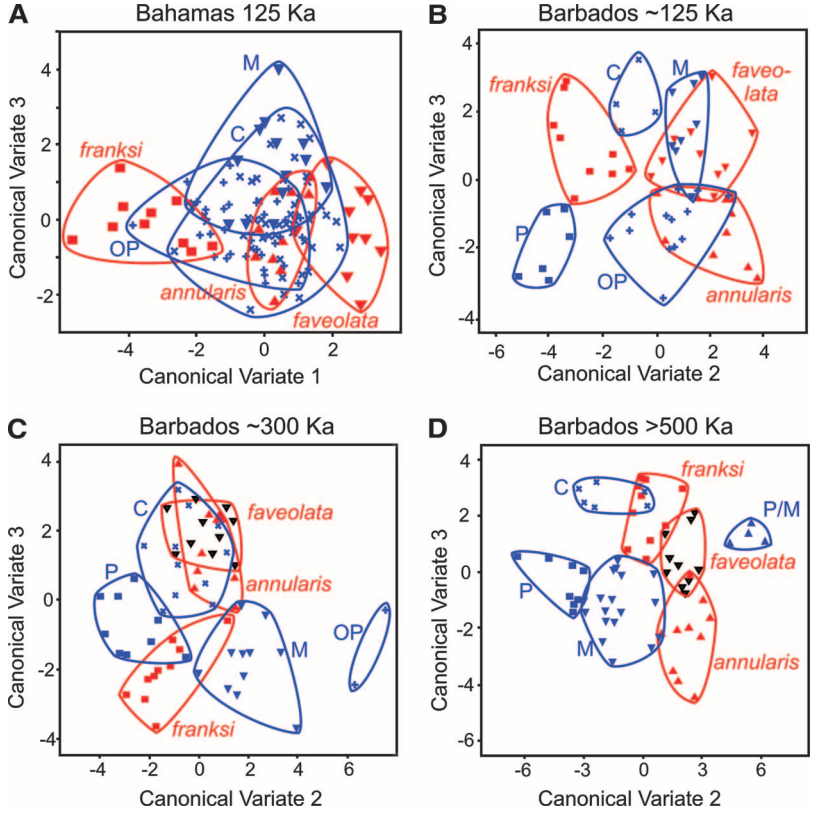

Fig. 1. Plots of scores on canonical variates comparing the three Recent species (red) with the fossil morphospecies (blue) aged (**A**) ~125 ka in the Bahamas, (**B**) ~125 ka in Barbados, (**C**) ~300 ka in Barbados, and (**D**) >500 ka in Barbados. The canonical variates show the maximum Mahalanobis distances among Recent Panama species along the *x* axis and fossil colony forms along the *y* axis (table S3). Each point represents one colony; polygons enclose the maximum variation within species or morphospecies. Correlations of canonical variates with original variables and other statistics are given in table S3. P, plate; M, massive; C, column; OP, organ-pipe; P/M, an additional massive species found only in the >500-ka Barbados assemblages.

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and *M. franksi*, which are morphologically and genetically distinct in Panama and Belize but not in the Bahamas (fig. S1). Morphological features that distinguish the three species include both colony growth form and various measures of corallite architecture (*18*, *22*). Genetic and reproductive variability have been identified at three amplified fragment length polymorphism (AFLP) loci, the noncoding region of the mitochondrial genome, and internal transcribed spacer sequences, along with differences in spawning time and gamete compatibility (*23*, *24*). *M. faveolata* and *M. franksi* have a fossil record extending back >2.5 million years and belong to different subclades (monophyletic units) within the complex, whereas *M. annularis* s.s. extends back only ~0.5 million years and is more closely related to *M. franksi* (*18*, *23*, *25*).

Our fossil samples consist of a total of 432 colonies that were collected from three groups of Pleistocene terraces on the island of Barbados [dated at ~640 to 860 thousand years ago (ka) (referred to here as >500 ka); at ~250 to 500 ka (~300 Ka); and at ~80 to 250 Ka (~125 ka)] and from late Pleistocene coastal terraces (~125 ka) at four other Caribbean locations (Table 1). Morphospecies were recognized within each of these seven fossil units by comparing colony growth forms (plate, massive, column, and organ-pipe) using geometric morphometric techniques (*17*) and multivariate statistical analyses (canonical variates analysis) of the coordinates of 19 landmarks digitized in transverse thin section. Samples within each fossil unit were first analyzed separately to distinguish morphospecies. Thirty-two modern genetically characterized colonies from the San Blas Islands of Panama (*23*) were included in each analysis to provide a baseline for morphospecies recognition. *F* values corresponding with Mahalanobis distances were used to determine whether any of the fossil morphospecies were the same as the three modern species. In the three Barbados fossil units, morphospecies were also analyzed with a combined canonical variates analysis, and the resulting Mahalanobis distances were used to trace lineages through geological time. Finally, the Mahalanobis distances between all pairwise combinations of morphospecies within each fossil unit were standardized and compared among units to test for differences in morphological disparity. These distances were used to create an index of evolutionary novelty, which is calculated for each locality using the product of its species richness times the deviation of its mean distance from the global mean distance (*26*). Evolutionary novelty indices increase as morphological diversity increases.

Speciation and extinction rates were consistently higher in Barbados than in the other four Caribbean locations (see numbers of first and last occurrences in Table 1). Three or four morphospecies were found within each of the seven fossil units, indicating that diversity remained the same in the complex both spatially and temporally during the frequent climatic oscillations of the late Pleistocene (Fig. 1; Table 1). In each of the seven fossil units, one morphospecies was the same as *M. faveolata*; in all of the fossil units except Barbados, another morphospecies was the same as *M. annularis* s.s. *M. franksi* was found only in the Dominican Republic. An extinct organ-pipe form (*M. nancyi*) first appears at ~125 ka and was found at all five ~125-ka sites (*27*). An additional morphospecies (an extinct platy form, P) could also be traced through the three time units in Barbados. The remaining morphospecies in Barbados, which consist of massive (P/M), columnar (C1, C2, and C3) and organ-pipe (OP) forms, do not match the three modern species or morphospecies in the other fossil units and appear to be unique. In contrast, the species composition at the other four Caribbean locations was exactly the same at ~125 ka and in Recent times. The wider geographic distribution found in *M. faveolata* than in *M. annularis* s.s. during the Pleistocene conforms with population genetic divergence estimates made using 10 nuclear DNA loci (7 microsatellite and 3 single-copy restriction fragment length polymorphism), which show higher gene flow and greater population connectivity in *M. faveolata* (*28*).

Mahalanobis distances between all pairwise combinations of morphospecies within each fossil unit were significantly lower in the ~125-ka terrace in the Bahamas and significantly higher in the three Barbados fossil units (Fig. 2A), as compared with the other Caribbean locations. Moreover, these two peripheral locations (Barbados and the Bahamas) had higher indices of evolutionary novelty than all other locations (Fig. 2B). In the Caymans, Dominican Republic, and Florida, Mahalanobis distances between fossil morphospecies were low and evolutionary novelty indices were intermediate, in comparison with those observed in modern species from Belize and Panama. The extremely low disparity (high species overlap) and high evolutionary novelty index observed in the Bahamas were interpreted as lineage fusion that resulted from introgressive hybridization within the species complex since 125 ka (*17*), an interpretation that agrees with genetic data (fig. S1). In the genetic analyses, hybridization was found in only one AFLP locus in one species (*M. annularis* s.s.) and in only 15% of the

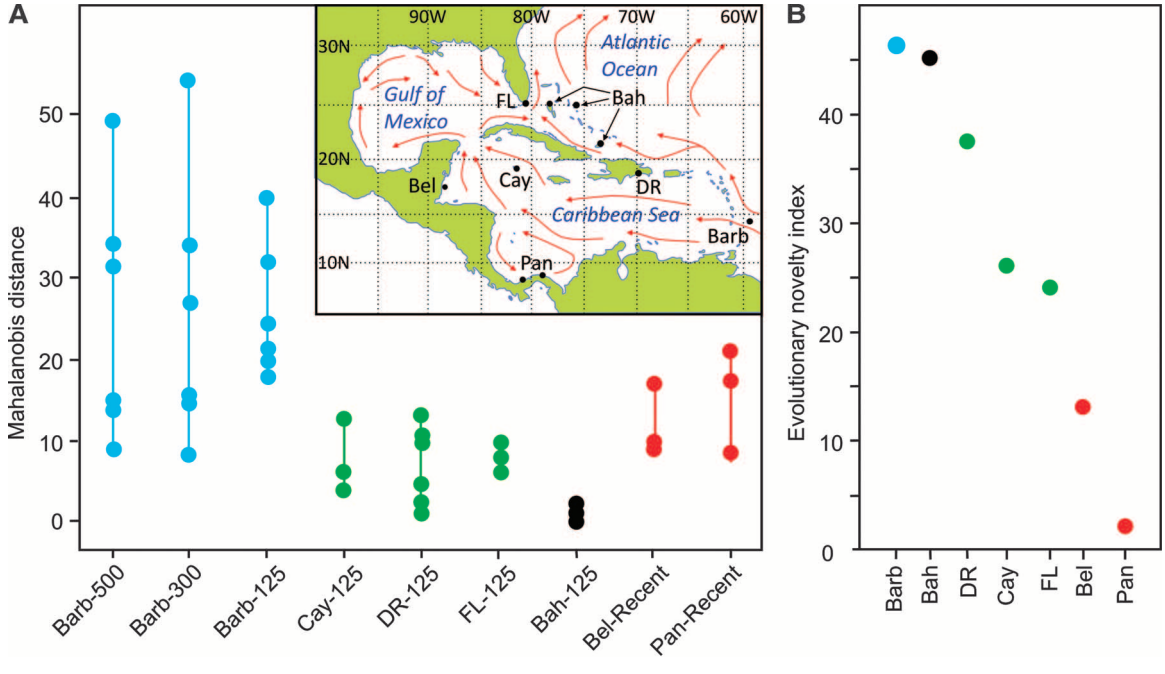

Fig. 2. Scatterplots showing (**A**) Mahalanobis distances between species at each locality (table S5) and (**B**) an index of evolutionary novelty defined as the product of species diversity and the absolute value of the deviation of the locality mean Mahalanobis distance from the global mean Mahalanobis distance. Mahalanobis distance values are high in the Pleistocene of Barbados (median = 23, blue), intermediate to high in the Recent of Belize and Panama (median = 13, red), intermediate in the Pleistocene of the central Caribbean (median = 8, green), and low in the Pleistocene of the Bahamas (median = 1, black). Mann-Whitney U tests performed on these distances show that the four groups are significantly different ($P \leq 0.05$).

Table 1. Summary of sampled morphospecies recognized within different fossil units. P, P/M, C1, C2, C3, and OP correspond with morphospecies shown in Figs. 1 and 2. The specimens that were analyzed are listed in appendix S1. na, not applicable.

Fossil unit	Total number of colonies sampled	Total number of morpho-species	Morpho-species with first occurrences	Morpho-species with last occurrences	Morpho-species corresponding with modern species	Extinct morpho-species that carry over to the next younger unit
Barbados >500 ka	36	4	P, P/M, C1	P/M, C1	*faveolata*	P
Barbados ~300 ka	33	4	C2, OP	C2, OP	*faveolata*	P
Barbados ~125 ka	32	4	C3, *nancyi*	P, C3, *nancyi*	*faveolata*	na
Cayman Islands ~125 ka	77	3	*nancyi*	*nancyi*	*faveolata, annularis* s.s.	na
Dominican Republic ~125 ka (*17*)	114	4	*nancyi*	*nancyi*	*faveolata, annularis* s.s., *franksi*	na
Florida ~125 ka	34	3	*nancyi*	*nancyi*	*faveolata, annularis* s.s.	na
Bahamas ~125 ka (*17*)	106	3	*nancyi*	*nancyi*	*faveolata, annularis* s.s.	na

colonies sampled, suggesting that this genetic signal may reflect an ancestral polymorphism rather than recent events (*23*, *24*). The significantly higher disparity observed in Barbados, where we do not have genetic data, may be interpreted as an evolutionary innovation (lineage splitting), which could have been caused by evolutionary mechanisms ranging from hybridization (resulting from increased gene flow between species) followed by ecological diversification, to parapatric or peripatric speciation (resulting from restricted or no gene flow between diverging peripheral populations). Studies of hybrids in land snails (*12*) have shown that novel morphologies may be caused by novel alleles or by the inheritance of a mosaic of morphological characters related to the geometry of shell coiling, which during growth can lead to novel adult morphologies. Hybridization could similarly lead to evolutionary novelty and adaptation in the *M. annularis* complex. Alternatively, evolutionary novelty could be caused by reproductive isolation associated with parapatric or peripatric speciation.

In addition to Caribbean members of the *M. annularis* complex, hybridization has been observed in modern Caribbean *Acropora*, but the hybrid *Acropora* morphotypes (*A. prolifera*) are not hybridizing species and have little evolutionary potential (*29*). In contrast, all three of the *M. annularis* species are vital and can be crossed under lab-controlled conditions, suggesting that they have the potential to hybridize. However, other pre- and post-mating isolation mechanisms must operate to maintain them as distinct species (*23*, *24*). Therefore, the outcome of hybridization is different between *Acropora* and *Montastraea* in the Caribbean.

Caribbean reef corals of the *M. annularis* species complex exhibited significant geographic differences in evolutionary response to Pleistocene climatic oscillations, despite the absence of the range shifts observed in many terrestrial organisms (*21*). Lineage splitting (Barbados) and fusion (the Bahamas) were concentrated at edge zones, which we hypothesize were characterized by limited larval supply and population connectivity, in contrast with well-connected interior locations near the Caribbean biodiversity center (the Dominican Republic, Cayman Islands, and Florida), which exhibited lineage stasis and had species whose morphologies are the same as those of modern species in Panama and Belize (Fig. 2A). Barbados apparently represents a source population from which larvae disperse, which lies along an edge of the geographic distribution of the complex, where currents first begin to move through the Caribbean and there are no extant external sources of larvae. The Bahamas were similarly isolated during the Pleistocene but represent a sink population into which larvae immigrate. During low Pleistocene sea level stands, larval flow through the Caribbean may have been diminished by restricted oceanic currents, and flow between the Bahamas platform and the rest of the Caribbean would have been limited. In addition, water movement across the Bahamas platform itself would have been reduced (*30*, *31*). The observed differences in evolutionary response between locations correspond with the genetic discontinuity in the Mona Passage between Puerto Rico and the Dominican Republic, which separates eastern from western Caribbean populations of reef fish and acroporid corals today (*32*, *33*). Moreover, empirical data for living *Acropora* also indicate that the Bahamas are isolated from the rest of the Caribbean (*33*).

Lower gene flow to edge locations would have altered population dynamics, thereby enhancing evolutionary innovation. In the Bahamas (the sink population), fewer immigrants would have led to hybridization during population decline, which resulted in genetic assimilation, reduced genetic variance, and overlapping morphology. In Barbados (the source population), geographic isolation would have led to genetic differentiation, causing evolutionary diversification and the creation of novel morphologies. One example of a speciation and range extension was *M. nancyi*, which may have arisen in Barbados as much as 250 ka and spread across the Caribbean before it ultimately became extinct between 82 and 3 ka (*25*, *27*).

Our work emphasizes the need to consider the fossil record in addition to genetic and physical data in order to obtain a more complete picture of factors influencing reef connectivity and evolutionary responses to environmental change. Our data suggest that species edge zones play an important role in evolutionary innovation, which may be caused by factors ranging from hybridization to parapatric or peripatric speciation, depending on population dynamics. These interpretations agree with recent results for reef fish (*34*) and hermit crabs (*35*). Edge zones are not only potential evolutionary cradles but are likely to be important sources of evolutionary innovation, especially as they migrate in the face of projected climate change. As such, we believe that species edge zones and peripheral areas, such as the eastern Caribbean, together with population connectivity, should play a prominent role in the future design (number, placement, and size) of marine reserves.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/328/5985/1558/DC1
Materials and Methods
Figs. S1 and S2
Tables S1 to S5
References
Appendix S1

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Identification of Germline Stem Cells in the Ovary of the Teleost Medaka

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Germline stem cells continually produce sperm in vertebrate testes, whereas there is no direct evidence showing that germline stem cells are present in adult vertebrate ovaries. By using transgenic methods and clonal analysis, we identified germline stem cells that supported oogenesis and the production of offspring in the ovaries of adult medaka fish. Early-stage germ cells were localized in clusters along interwoven threadlike cords of *sox9b*-expressing somatic cells (termed germinal cradles) where the germ cells developed. Germline stem cells gave rise to germ cells that divided to produce cysts, which then underwent cell death or separated to form follicles. Our results provide insight into the germline stem cell biology of medaka and provide a model system for studying vertebrate stem cell niches.

Many organisms continually produce gametes from germline stem cells in specific niches within the testis (*1*). In contrast to the testes, the ovaries of most adult mammals contain only postmitotic germ cells and a finite number of mature eggs. Only a few examples of mammals are suggested to have stem-like germ cells and mitotic oogonia in adult ovary, which, however, remain controversial (*2*–*6*). Nevertheless, some species—including lower vertebrates that show high fecundity—may produce oocytes either cyclically or continually from mitotic oogonia (*7*–*9*). A stem cell–based mechanism and histological evidence to support this mechanism, however, have not been shown in adult ovaries.

Many studies have identified genes involved in gonadal sex differentiation. *Sox9*, for example, is essential for mammalian testicular development (*10*, *11*). In medaka (*Oryzias latipes*), a teleost fish, *sox9b* is expressed in the developing gonads, where it is thought to be the functional ortholog of mammalian *Sox9* (*12*).

We used EGFP (enhanced green fluorescent protein) fluorescence to visualize endogenous *sox9b*-expressing cells in the ovaries of transgenic adult medaka (*sox9b*-EGFP medaka) (*13*); the *sox9b*-expressing cells formed interwoven threadlike cords (Fig. 1, A and B, and fig. S2, A to D). Early germ cells were detected by using germ line–specific markers, OLVAS [medaka (*Olyzias latipes*) vasa] and tdrd1 (tudor domain containing 1) (*14*–*16*), components of a germ line–specific intracellular structure called nuage. These cells were nested in the cords of *sox9b*-expressing cells (Fig. 1B) and were connected by thin processes originating from the *sox9b*-expressing cells. Expression of *sox9b* by cells surrounding the germ cells in the ovary was confirmed by using in situ hybridization (fig. S2E). Hereafter, we refer to the nest of germ cells in the cord as the germinal cradle in the ovarian cord. Laminin staining (Fig. 1, C and D), three-dimensional tissue imaging (fig. S2F and movie S1), periodic acid–Schiff (PAS) staining (fig. S2, G and H), and electron micrographs (fig. S2, I and J) showed that the ovarian cords and germinal cradles were buried in the germinal epithelium, a thin multilayered tissue that covers the dorsal side of the stromal compartment (fig. S1, A and B). The germinal cradles lay between epithelial cells of the germinal epithelium and the basement membrane bordering the stromal compartment. Germinal epithelia from 3-month-old adult medaka contained 629 ± 22 germinal cradles (mean ± SEM; $n = 4$).

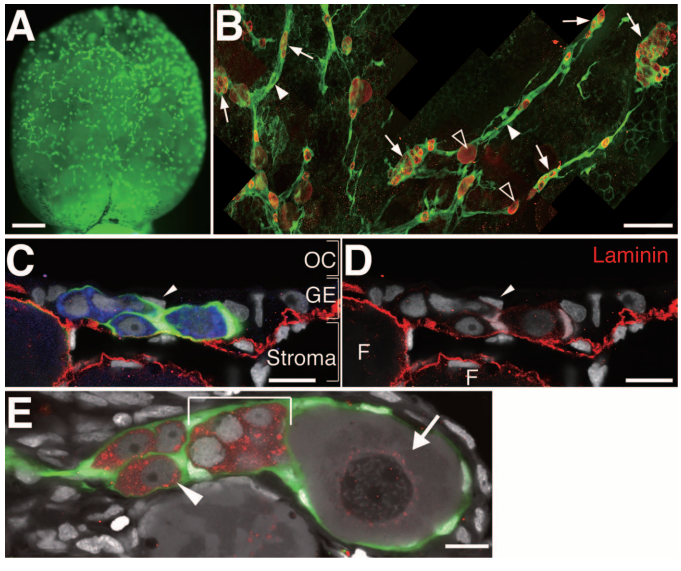

Fig. 1. Ovarian cords in adult medaka are composed of early-stage germ cells and *sox9b*-expressing cells. Scale bars indicate 1 mm (A), 100 μm (B), or 10 μm [(C) to (E)]. (**A**) A dorsal fluorescent image of an ovary from a *sox9b*-EGFP–expressing adult medaka. (**B**) An enlarged image of an immunostained ovary. Germ cells detected on the basis of OLVAS expression (red) are nested in germinal cradles (arrows) connected by cellular processes (arrowheads) that originate from *sox9b*-EGFP–expressing cells (green). Open arrowheads, isolated diplotene oocytes. (**C** and **D**) A cross section of the germinal epithelium. A germinal cradle composed of *sox9b*-expressing cells (green) and early-stage germ cells (blue, OLVAS) lies between dorsal epithelial cells (arrowheads) and basement membrane (red, laminin). OC, ovarian cavity; GE, germinal epithelium; Stroma, stromal compartment; F, follicle. Gray, 4′,6′-diamidino-2-phenylindole (DAPI). (**E**) A representative germinal cradle. Arrowhead, isolated Gs cells; bracket, Gcys cells; arrow, early-stage Gdip oocytes. Germ cells expressing tdrd1, red; *sox9b*-expressing cells, green; DAPI, gray.

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