Recent speciation in the Indo-West Pacific: rapid evolution of gamete recognition and sperm morphology in cryptic species of sea urchin

C. Landry[1], L. B. Geyer[1]†, Y. Arakaki[2], T. Uehara[3] and Stephen R. Palumbi[1]*†

[1]*Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA*
[2]*Department of Tourism, Faculty of International Studies, Meio University, Nago, Okinawa, Japan*
[3]*Department of Biology, University of the Ryukyus, Nishihara, Okinawa, Japan*

The rich species diversity of the marine Indo-West Pacific (IWP) has been explained largely on the basis of historical observation of large-scale diversity gradients. Careful study of divergence among closely related species can reveal important new information about the pace and mechanisms of their formation, and can illuminate the genesis of biogeographic patterns. Young species inhabiting the IWP include urchins of the genus *Echinometra*, which diverged over the past 1–5 Myr. Here, we report the most recent divergence of two cryptic species of *Echinometra* inhabiting this region. Mitochondrial cytochrome oxidase 1 (CO1) sequence data show that in *Echinometra oblonga*, species-level divergence in sperm morphology, gamete recognition proteins and gamete compatibility arose between central and western Pacific populations in the past 250 000 years. Divergence in sperm attachment proteins suggests rapid evolution of the fertilization system. Divergence of sperm morphology may be a common feature of free-spawning animals, and offers opportunities to simultaneously understand genetic divergence, changes in protein expression patterns and morphological evolution in traits directly related to reproductive isolation.

Keywords: speciation; bindin; Okinawa

1. INTRODUCTION

The tropical shelf waters of the Indo-West Pacific (IWP) house a rich species diversity that has its zenith around the East Indies. Species richness of many taxa increases from the eastern Pacific and the Indian Ocean towards the Indonesian Archipelago (reviewed in Briggs 1999; Bellwood & Hughes 2001). Although previous investigations of the genesis of this diversity emphasized the slow accumulation of species over long periods of time (Springer & Williams 1990), recent genetic evidence suggests that many of the reef species in this diverse area are relatively new, having originated since the beginning of the Pleistocene (Palumbi 1996; McMillan *et al.* 1999; Barber *et al.* 2002). These recent speciation events provide a laboratory in which to understand the speciation process of marine taxa, and allow a combination of genetic, morphological, behavioural and biogeographic data to be used to answer questions about speciation mechanisms. Among others, allopatry, peripheral speciation and divergence after secondary range contact have been proposed as drivers of species diversity in the IWP (Palumbi 1997; Bellwood & Hughes 2001), but as yet there have been very few clear reconstructions of speciation history of marine taxa in this area.

Footprints of species formation are most likely to be identified when comparing recently diverged species, initial differentiation of which can be correlated with the different proposed speciation processes. Molecular tools have helped to uncover closely related and still diverging

species from a wide variety of taxa and have provided insight into the mechanisms, pace and geography of marine speciation (Knowlton 1993; Palumbi 1997; Lessios *et al.* 2001). Coupled with information about geological history of regions, faunistic affiliations of species found in particular regions, and the oceanic currents that may provide dispersal corridors, molecular data can greatly help document the geographic pattern and relative timing of species formation.

Among the closely related species that help contribute to the richness of the IWP are the sea urchins of the genus *Echinometra*. Pan-tropical sea urchins of the genus *Echinometra* diverged into two different evolutionary lineages 3.3–4.5 Myr ago when the Indo-Pacific (IP) lineage separated from the one leading to the neotropical species (McCartney *et al.* 2000). Since then, the IP species lineage has undergone several speciation events. Molecular phylogenetic analyses, morphological studies and fertilization experiments have been combined to map the boundaries of four closely related *Echinometra* species common throughout the tropical Pacific (see Mortensen 1943; Uehara *et al.* 1986; Matsuoka & Hatanaka 1991; Palumbi & Metz 1991; Metz *et al.* 1994; Palumbi 1996; Arakaki *et al.* 1998*a*,*b*).

Among these is *Echinometra oblonga*, a black species that occupies reefs from Mauritius in the Indian Ocean to Isla del Coco in the eastern Pacific (figure 1). Typically, this species inhabits burrows above the mean low water level, in microhabitats that are highly exposed to wave action (Keslo 1970; Nishihira *et al.* 1991). This habitat preference correlates with species-specific physiological adaptations providing resistance to extreme conditions such as high and low temperatures, salinity changes (Arakaki & Uehara 1991) and strong shearing action of waves on

*Author for correspondence (spalumbi@stanford.edu).
† Present address: Department of Biological Sciences, Stanford University, Hopkins Marine Station, Pacific Grove, CA 93950, USA.

Proc. R. Soc. Lond. B (2003) **270**, 1839–1847
DOI 10.1098/rspb.2003.2395
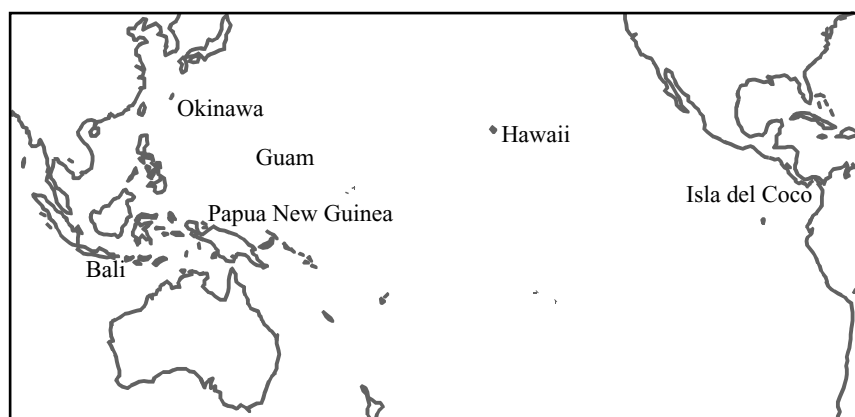
1839

© 2003 The Royal Society

Figure 1. Schematic representation of collecting localities in the Pacific Ocean for *Echinometra oblonga* populations. Western Pacific: Bali (Indonesia), Okinawa (Japan) and Papua New Guinea; central Pacific: Guam (US), Hawaii (US) and Isla del Coco (Costa Rica).

gametes (Mead 1996). This species has been described from Japan and Hawaii on the basis of monophyletic mitochondrial DNA (mtDNA) lineages (Palumbi & Metz 1991), allozyme differences (Matsuoka & Hatanaka 1991), reproductive incompatibility with sister species (Uehara *et al.* 1990; Palumbi & Metz 1991; Metz *et al.* 1994) and large differences in the sperm protein bindin that attaches sperm to eggs before fertilization (Metz & Palumbi 1996). This species is among a complex of at least three other species that occur commonly throughout the IWP, and have been examined closely for population structure (Palumbi *et al.* 1997) and mechanisms of speciation (Palumbi 1999).

Careful analysis of data on *E. oblonga* from numerous localities throughout its range has shown marked polymorphism. For instance, individuals from Mauritius, Okinawa, Bonin, Guam and Hawaii differ slightly, although not diagnostically, in the number of pore pairs, as well as the shape of the spicules of the gonads and tube feet (Arakaki & Uehara 1999). Furthermore, these characteristics are not consistent with each other within *E. oblonga*: some characteristics cluster populations of Okinawa and Hawaii together and Indonesia and Guam together, whereas others differentiate the Hawaiian population from the rest of the Pacific (Arakaki & Uehara 1999). Another difference across the Pacific is sperm morphology: western Pacific populations have a sperm head that is twice as long as those from the central Pacific (figure 4; Arakaki & Uehara 1999). Recently, some central and western Pacific populations have been shown to be differentiated at bindin, a sperm–egg recognition protein (Geyer & Palumbi 2003). These results suggest that populations of *E. oblonga* may actually represent diverging species that differ in reproductive characteristics such as bindin sequence and sperm morphology. However, genetics, sperm morphology and gamete attachment features have never been examined in the same suite of populations, making delineation of biogeographic boundaries for these potential species difficult, and obscuring potential insights into the process of speciation.

In this study, our aims are to characterize populations of *E. oblonga* for two genetic loci and for sperm morphology to test if the differences observed reflect the reproductive isolation of these populations and to discuss the history and the geography of this diversification. We investigate the phylogenetic relationship among *E. oblonga* populations from the western and central Pacific and compare genetic data with morphological differences. Because of their large population size and their recent divergence, *Echinometra* species are expected to be monophyletic at very few of their loci. However, mitochondrial genomes are expected to drift faster than nuclear genes and are then expected to show a signal of differentiation earlier in the speciation process (Palumbi *et al.* 2001). Previous work on mitochondrial sequences provided only slight evidence of genetic separation between *E. oblonga* populations based on the segregation of a single polymorphic site (Palumbi *et al.* 1997). Here, we increase the sequence length to provide more resolving power, and include additional populations that have been investigated for both sperm and bindin features. To put mtDNA diversity data within species into a larger perspective, we also include in our analysis other closely related species found in the IP (*E. mathaei*, *E.* sp. C and *E.* sp. A) and a sister species endemic to Easter Island (*E. insularis*). *Echinometra vanbrunti*, a congeneric species found in the eastern Pacific is used as an outgroup.

2. METHODS

Populations of *E. oblonga* were sampled from Isla del Coco ($n = 2$), Hawaii ($n = 5$), Guam ($n = 5$), Okinawa ($n = 11$), Bali ($n = 11$) and Papua New Guinea ($n = 3$). We included 15 samples of *E. mathaei*, three of *E.* sp. A, 14 of *E.* sp. C and 10 of *E. insularis*. DNA was extracted from tube feet, gonad or spine muscle tissue by proteinase K digestion and phenol/chloroform extraction, or by using NucleoSpin columns (BD, Biosciences, Clonetech).

Two overlapping fragments of the cytochrome oxidase 1 (CO1) gene corresponding to the interval between sites 5851 to 7013 of *Strongylocentrotus purpuratus* mitochondrial genome were sequenced for each individual. Samples corresponded in part to the samples analysed for bindin by Geyer & Palumbi (2003). The 3′ fragment was amplified and sequenced by using primers CO1f and CO1d according to Palumbi *et al.* (1997). The 5′ fragment was obtained by using primers LCO1490 and HCO2198 (Folmer *et al.* 1994) by using the same chemistry and the following cycles: 94 °C 30 s; 50 °C 45 s; and 72 °C 1 min,
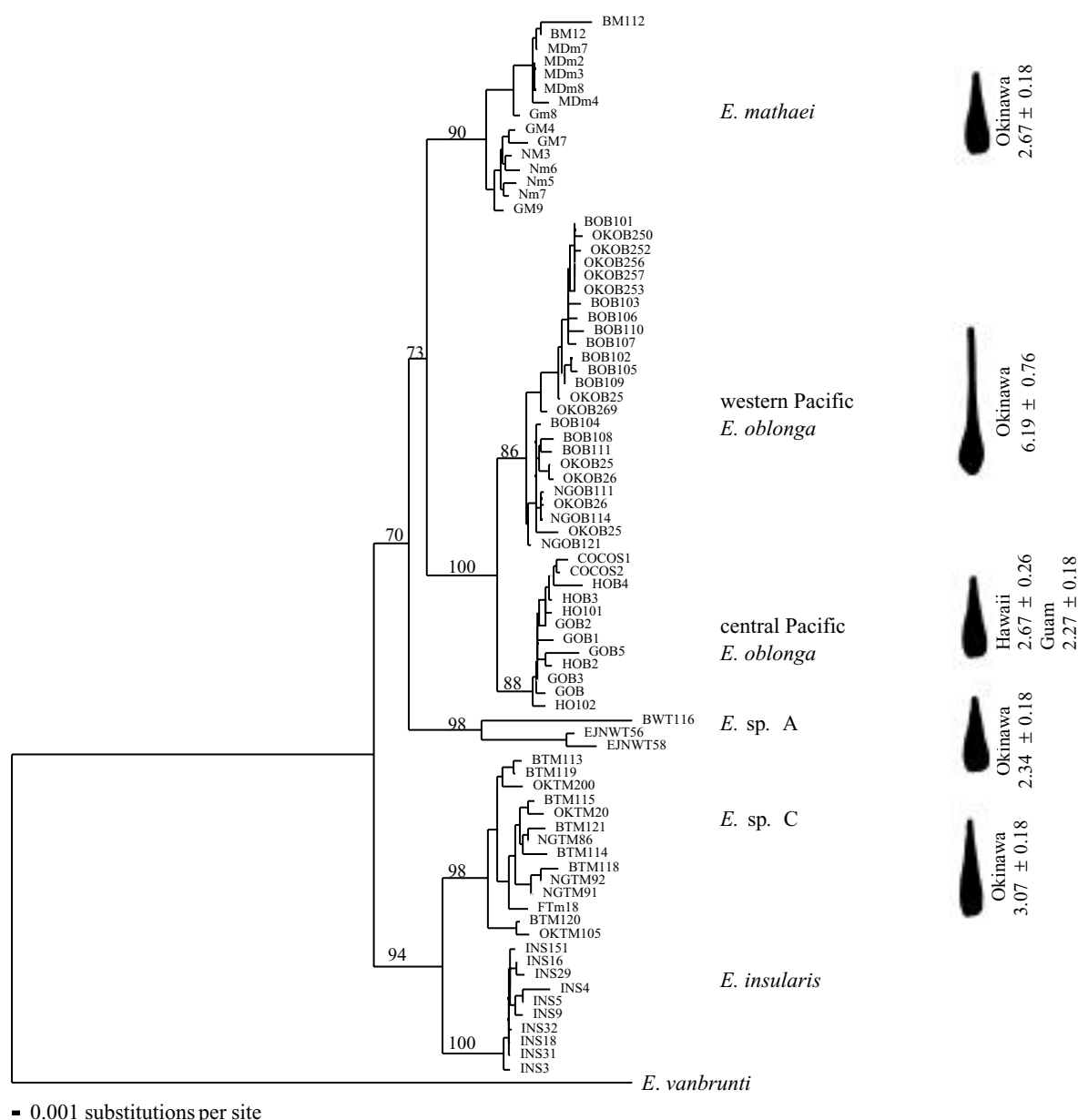
Figure 2. Rooted NJ tree of CO1 sequences (K_2 distance). Bootstraps were carried out by resampling all nucleotide positions 1000 times. The first one or two first letters of the sample names represent the sampling locality. (F, Fiji; OK, Okinawa; NG, Papua New Guinea; B, Bali; H, Hawaii; Co, Isla del Coco; M, Midway, Hawaii; N, Nieu; EJN, Japan; INS, Easter Island). Sperm morphologies are schematized and lengths (micrometres) are from Arakaki *et al.* (1998*b*) and Arakaki & Uehara (1999).

and a final elongation of 5 min at 72 °C. Including the primers, the two fragments overlap by 66 bp. Polymerase chain reaction (PCR) products were prepared for sequencing by using Exonuclease I and shrimp alkaline phosphatase digestion and were then sequenced on a 3100 ABI automated sequencer (Applied Biosystems, CA, USA). Sequences were aligned by hand. Within-species and among-species polymorphisms of CO1 sequences were estimated by using MEGA (Kumar *et al.* 2001). The net average distance corresponds to the average distance minus the average within-species polymorphism. The phylogenetic relationship of the samples was drawn by using the neighbour-joining (NJ) algorithm from PAUP v. 4.0 (Swofford 2001).

Bindin alleles were amplified, cloned and sequenced as previously described (Palumbi 1999; Geyer & Palumbi 2003) from individuals collected in Okinawa ($n = 4$), Bali ($n = 14$), Guam ($n = 6$), Hawaii ($n = 11$) and Niue ($n = 8$). A total of three to five clones from each individual were sequenced. When two alleles were encountered, both were included in the analysis. Otherwise, a single allele was analysed from each individual.

3. RESULTS

A portion of the mitochondrial CO1 gene corresponding to bases 5851 to *ca.* 6550 of the *S. purpuratus* complete mitochondrial genome was obtained for 79 individuals of five putative IWP species of *Echinometra* (GenBank accession numbers AY262861–AY262940). When added to sequences already available for these individuals, our dataset comprises 1164 bp of the CO1 gene.

Most of the substitutions within *Echinometra* are at silent sites in this coding sequence, and the

Table 1. Between-species average K_2 distance (below the diagonal) at all sites (pairwise deletion) and net average distance (above the diagonal). Average intraspecific nucleotide diversity (π) is shown on the diagonal (in italics).

	1	2	3	4	5	6	7
Echinometra oblonga western Pacific	*0.004*	0.009	0.033	0.027	0.022	0.034	0.132
E. oblonga central Pacific	0.013	*0.003*	0.034	0.027	0.019	0.036	0.133
E. sp. C	0.039	0.039	*0.007*	0.030	0.030	0.015	0.127
E. sp. A	0.040	0.040	0.046	*0.021*	0.021	0.029	0.127
E. mathaei	0.027	0.024	0.037	0.034	*0.006*	0.030	0.126
E. insularis	0.037	0.038	0.019	0.040	0.034	*0.002*	0.129
E. vanbrunti	0.135	0.134	0.131	0.138	0.129	0.130	*0*

transition/transversion ratio is about 11 : 1. We observed two amino acid changes in two sequences. Phylogenetic analysis of mtDNA sequences from 80 individuals revealed five major clades (figure 2) defined by high bootstrap values (90–100%). Four of these clades correspond to the four currently recognized IP species of *Echinometra* (Palumbi 1996). The clade including *E. oblonga* shows two additional, well-defined sub-clades, corresponding to *E. oblonga* from the central Pacific (Hawaii, Isla del Coco and Guam) and those from western Pacific populations (Indonesia, Okinawa and Papua New Guinea). These two clades are differentiated by seven fixed transition substitutions. The same organization among clades was obtained by using a parsimony analysis (C. Landry, unpublished data).

Using *E. vanbrunti* as an outgroup (McCartney *et al.* 2000), the IP *Echinometra* fall into two multi-species clades. One contains *E.* sp. C, a species found among few Pacific island archipelagoes (Palumbi 1996), and *E. insularis*, which is endemic to Easter Island. The other clades contain the widely distributed *E. mathaei*, *E. oblonga* and *E.* sp. A. Bootstrap support for the branching order of these three species is poor, but data from both morphology and bindin sequences suggest a sister-species relationship between *E. mathaei* and *E.* sp. A. Within this cluster, the two clades of *E. oblonga* are derived sister taxa (figure 2).

Average K_2 parameter distance (where K_2 is the silent rate at twofold degenerate nucleotide positions; Kumar *et al.* 2001) among IWP *Echinometra* species considering all the sites (pairwise deletion) ranges from 1.3% to 4.6%, which is very similar to the distances measured using only the CO1f-a gene fragment (Palumbi 1996; McCartney *et al.* 2000). Mean K_2 parameter distances to the eastern Pacific *E. vanbrunti* vary between 12.9% and 13.8%, which is slightly higher than was calculated by McCartney *et al.* (2000) for the smaller gene segment. Variation within species ranges from 0.2% to 2.1% for these samples (table 1).

The average genetic distance between the two groups of *E. oblonga* (1.3%; net distance: 0.9%) is low compared with the 4.0–4.8% difference between *E.* sp. A, *E.* sp. C and *E. insularis*, but is comparable to the distance between *E.* sp. C and the endemic *E. insularis* (1.9%). Under the assumption of constant rates of CO1 evolution, it is possible to use divergence data between mitochondrial sequences to estimate the time of the split between *E. oblonga* clades. Using the average sequence divergence at CO1 between species of *Echinometra* found on both sides

of the Isthmus of Panama, McCartney *et al.* (2000) derived an estimate of 3.49% Kimura-corrected sequence divergence per million years, with a range that spans 24% of this mean. Using this rate, we estimated a divergence time between the two types of *E. oblonga* (net $K_2 = 0.9\%$, s.e.m. 0.5%) of *ca.* 250 000 years.

(a) *Bindin sequences*

Central and western Pacific populations of *E. oblonga* are characterized by very divergent bindin alleles. Bindin sequence diversity among all *E. oblonga* populations combined is much higher (2.5%, average corrected p-distance) than in any of the other IP *Echinometra* species (1.6%, *E. mathaei*; 0.9% *E.* sp. A, 0.8% *E.* sp. C). Division of the data into central and western Pacific populations explains 55% of the variation observed. Central Pacific *E. oblonga* (Nieu, Guam, Hawaii) have bindin sequences that fall into three major clades (clades one, two and four; figure 3). By contrast, bindin from western Pacific *E. oblonga* (Indonesia, Okinawa and Papua New Guinea) fall in a distinct clade separated from the rest by unique insertion and deletion events, plus amino acid substitutions (clades three a and three b; figure 3). Clade three alleles are found almost exclusively in the west Pacific (Bali, Okinawa, New Guinea). The only exception was a single individual from Niue which was homozygous for this allele, but carried a central Pacific CO1 haplotype.

(b) *Sperm morphology*

Two different morphologies typify sperm from western and central Pacific populations. The western Pacific type has a slender sperm head, with a ratio of length over width of about six, whereas the central Pacific type has a shape more compact, with a ratio of length over width of about three (figure 4). The primary difference lies in the tip of the sperm head and the shape of the nucleus (Arakaki *et al.* 1998*a*,*b*). Populations of *E. oblonga* from Okinawa show the sperm morphology of western Pacific populations. Populations in Guam and Hawaii both have the shorter, stouter sperm morphology.

4. DISCUSSION

The species *E. oblonga* appears to have recently diverged into two species distinguishable by sequences of mitochondrial genes, genes for gamete recognition, and by sperm morphology. Data from CO1 show that the species *E. oblonga* contains two major monophyletic clades that differ from one another by 0.9% and that divide the
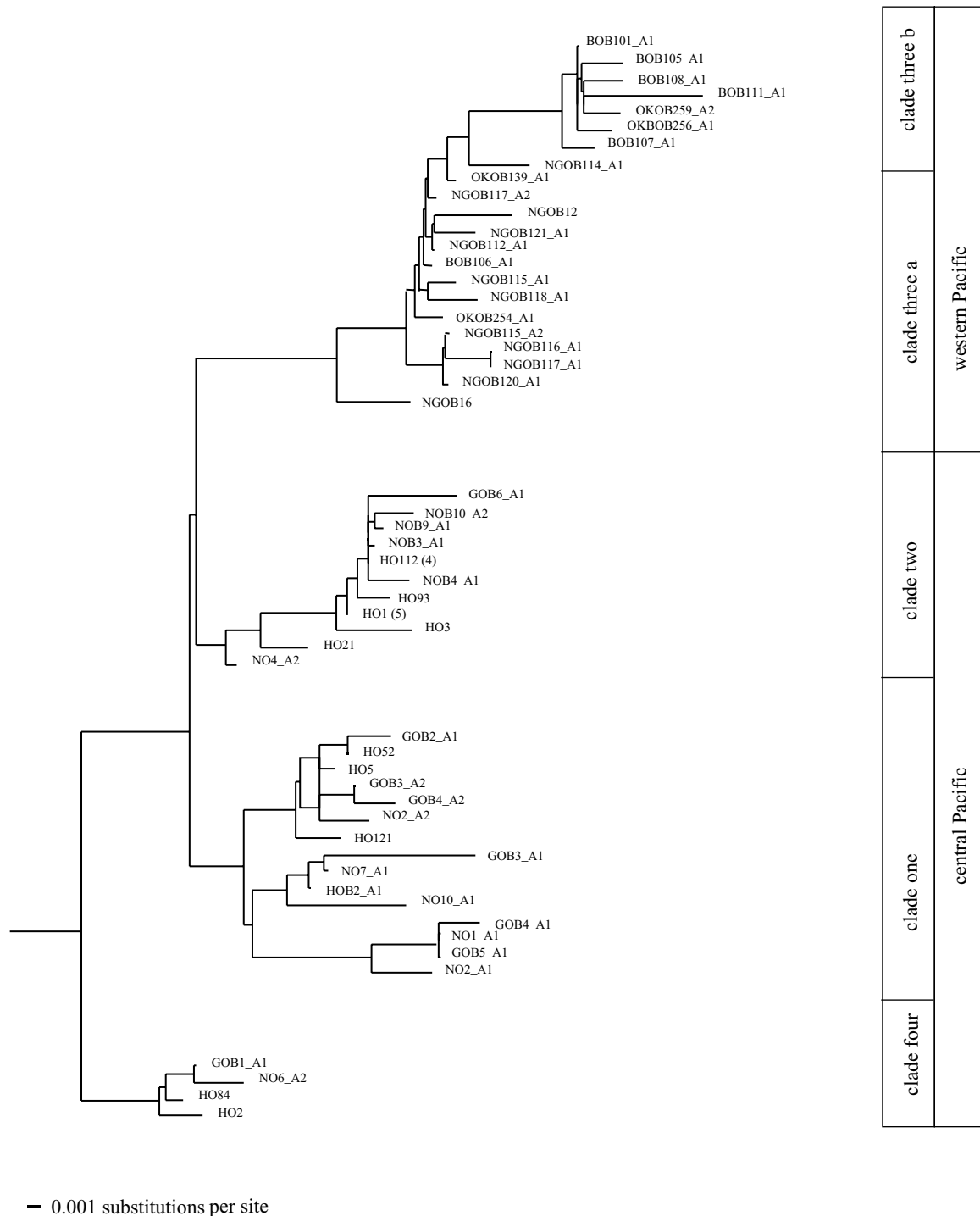
Figure 3. Divergence of bindin sequences in western and central Pacific populations of *Echinometra oblonga*. Sequences from the central Pacific fall into three major clades, all of which differ from the bindins seen in western Pacific populations. The branch leading to the western Pacific clades shows amino acid substitutions and insertions and deletions in the region of the protein implicated in functional sperm–egg attachment differences.

tropical Pacific into central and western areas. However, other sea urchin species in this genus harbour old mitochondrial lineages (Palumbi 1996), and so the mere existence of a significant split between central and western populations in CO1 does not guarantee species distinction. Instead, corroborative data are needed from other loci or organismal features before these data can be used to confirm species status of central versus western Pacific populations. Data from three other sources show agreement with this geographic pattern.

(a) *Bindin gene sequences and fertilization barriers*

The genetic differentiation seen in mtDNA is paralleled by differences at the gamete recognition locus bindin. Central Pacific populations differ strongly in bindin sequence from western Pacific populations, including amino acid and insertion/deletion changes in the protein coding region. The region of the bindin gene where these changes occur is the previously identified evolutionary hotspot where the action of positive diversifying selection
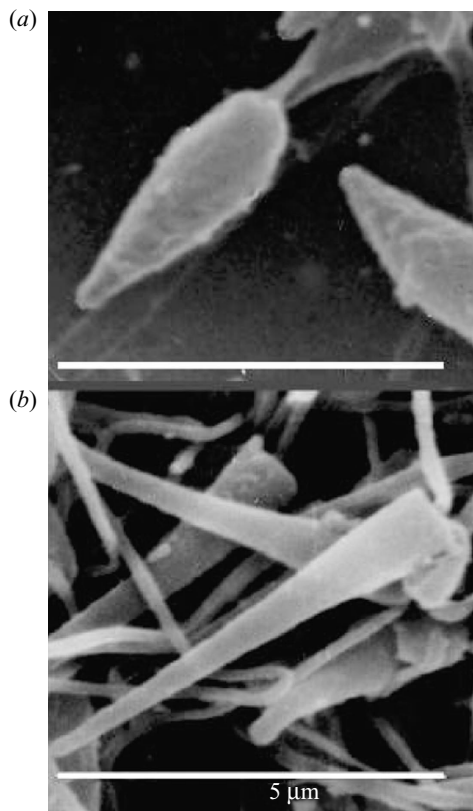
(a)

(b)

5 μm

Figure 4. Electron micrograph of sperm of *Echinometra oblonga* from (a) Guam and (b) Okinawa showing the difference in sperm length and shape.

has been observed (Metz & Palumbi 1996; Palumbi 1999). Bindin is involved in the attachment of the sperm to the egg, before its entrance into the egg, and initiates the first steps of fertilization (Vacquier & Moy 1977). This protein has been shown to influence fertilization success within species and has been proposed to participate in reproductive isolation among species (Metz *et al.* 1994; Palumbi 1999). As a result, these bindin gene changes are likely to have functional significance and could change egg–sperm attachment properties.

In fact, a reproductive barrier was suggested by recently reported cross-fertilization experiments between *E. oblonga* from Okinawa versus Guam and Okinawa versus Hawaii. Fertilization rates were high in control crosses, but were near zero for crosses between central and western populations (Arakaki 2000). Experiments with Indonesian or Papua New Guinean individuals have not been performed, and more extensive cross-fertilization work between populations should be completed. However, within-species and between-species variation in fertilization success owing to bindin differences has been demonstrated in other *Echinometra* species (Palumbi 1999) and as the western and central Pacific do not share bindin alleles, the same mechanism could explain the fertilization differences between *E. oblonga* populations.

(b) *Sperm morphology*

Sperm morphology among most species of *Echinometra* is highly conserved, with sperm heads about twice as long as wide, and central Pacific *E. oblonga* (Hawaii and Guam) have a typical *Echinometra* sperm shape (figures 2 and 4).

In marked contrast, spermatozoa of western Pacific populations (Okinawa, Indonesia and Mauritius) have a long anterior process, and are up to six times longer than they are wide (figure 4; Arakaki & Uehara 1989; Arakaki 2000). As with other mass-spawning invertebrates, sea urchins are known to have little premating behaviour or communication (e.g. Lamare & Stewart 1998). Especially when habitats and spawning seasons may overlap such as in *Echinometra* (Arakaki & Uehara 1991), gametic incompatibility is likely to be an important prezygotic mechanism of isolation (Palumbi 1992). Accordingly, the properties of sperm and eggs may often diverge during species formation, or function as the most proximal characteristics, ensuring the integrity of the species boundaries after secondary contact following allopatric divergence (Geyer & Palumbi 2003).

Sperm size in sea urchins can be polymorphic among populations within species (as in *S. droebachiensis*; C. Biermann, personal communication) but variation in length more than twofold usually represents differences among species (Chia *et al.* 1975; Amy 1983; Raff *et al.* 1990). In the present case, the two divergent sperm morphologies occur across the same geographical boundaries as genetic differences in mtDNA and bindin, and have not been reported to be polymorphic within populations (Arakaki 2000). The unique sperm morphology of the western Pacific *E. oblonga* suggests that this population exhibits a derived state that evolved in this region.

(c) *Gamete divergence and sympatry*

These concordant results of mtDNA differences, bindin sequence divergence, sperm morphology and fertilization suggest that the two populations of *E. oblonga* in the central and western Pacific have diverged into two sibling species. Although they each deserve a species name, the origin of the *E. oblonga* type specimen is not clearly known and therefore attributing a new species name to either the central or western Pacific populations will require more investigation.

The state of the genetic and morphological traits of the western Pacific *E. oblonga* suggests that it is the derived species. Factors driving the divergence of western Pacific *E. oblonga* are as yet unclear. However, the occurrence of slender sperm morphology and bindin sequence differences are correlated with the presence of *E.* sp. C, a species that generally occupies similar habitats on the western Pacific reefs (Nishihira *et al.* 1991; Uehara *et al.* 1996; Geyer & Palumbi 2003). This pattern is clearest in Guam. Guam is closer to Okinawa than Hawaii (*ca.* 2200 km versus *ca.* 7500 km), and shares many marine species (such as acroporid corals) with Okinawa and the rest of the IP that are absent in Hawaii. Even the echinometrid *E.* sp. A is shared between Guam and Okinawa, but is absent in Hawaii. On this basis, the black urchin in Guam was expected to be the western Pacific *E. oblonga*, but genetic and sperm traits consistently place it within the central Pacific *E. oblonga*. Despite the overall biogeographic similarity of Guam and Okinawa, Guam and Hawaii also share at least one biogeographic feature: they both lack *E.* sp. C.

When they co-occur, *E. oblonga* has been reported to occupy stronger wave action areas than *E.* sp. C (Arakaki & Uehara 1991). The derived sperm morphology might then be an adaptation to this habitat of higher wave

action. This hypothesis is supported by the similar elongated sperm morphology encountered in *Colobocentrotus mertensii* (Arakaki & Uehara 1989), another member of the family Echinometridae inhabiting high wave-action areas. It is also interesting that the sperm head of Okinawan *Echinometra* species increases in length in the order of *E.* sp. A < *E. mathaei* < *E.* sp. C < western Pacific *E. oblonga* but decreases in width in the same order, which correlates with the relative exposure of these species to wave action (Nishihira *et al.* 1991; Arakaki *et al.* 1998a,b; Mita *et al.* 2002). Longer sperm may not directly perform better in higher wave action but could be better at fertilizing bigger eggs, as proposed by Raff *et al.* (1990), which in turn could be better and more resistant in higher wave action. Data on egg size have not been reported for all the species but *E.* sp. A has been reported to have a smaller egg diameter than *E.* sp. C, which has a smaller egg diameter than western Pacific *E. oblonga* ($66.9 \pm 1.3 \,\mu m$ versus $71.8 \pm 1.4 \,\mu m$ versus $74.0 \pm 0.9 \,\mu m$) (Aslan *et al.* 1999; Rahman *et al.* 2001).

(d) *Speciation in* Echinometra

Along with previous reports, our results allow us to propose a history of this recent speciation event. Based on the derived genetic state of western Pacific *E. oblonga*, combined with derived sperm type, it appears that *E. oblonga* evolved in allopatry in the central Pacific, diverging from an ancestral stock common in the western Pacific within the past 0.5–1.0 Myr. *Echinometra oblonga* subsequently reinvaded the western Pacific, where it encountered the ancestral species *E.* sp. C, causing rapid changes in sperm morphology and gamete binding proteins. These changes resulted in gametic differences and reproductive barriers between *E. oblonga* currently found in the central Pacific and the new species in the western Pacific. Molecular clock estimates of divergence suggest that this reinvasion and speciation has taken place within the past 250 000 years.

(e) *Sperm and speciation*

Sex and speciation have been bound intimately in the definition of the biological species concept. Evidence is now accumulating that sexual selection might be acting when species are diverging and that within-species sexual selection may drive the development of reproductive isolation (reviewed in Panhuis *et al.* (2001)). Furthermore, there is ample evidence that sex-related genes are evolving quickly in a broad range of organisms (e.g. *Drosophila*, Civetta & Singh 1995; *Chlamydomonas*, Ferris *et al.* 1997; primates, Wyckoff *et al.* 2000; *Arabidopsis*, Mayfield *et al.* 2001; and marine invertebrates, Metz & Palumbi 1996). These rapidly changing genes may be correlated with the rapid evolution of genitalia and gamete morphology reported in many species. Some evidence suggests that sperm and reproductive organs are indeed rapidly evolving. For instance, in insects, genitalia are often the first morphological characters to change, which is why they are often used in systematics for the differentiation of closely related species (reviewed in Shapiro & Porter 1989). Sperm shape is also known to be rapidly evolving (e.g. Joly *et al.* 1991). In many species, sperm size has been shown to be related to male fitness (see, for example, Radwan 1996) and to respond to selection (e.g.

Gomendio & Roldan 1991; LaMunyon & Ward 2002). Spermatozoa in broadcast spawners are simultaneously both gametes and unicellular genitalia and therefore are expected to evolve quickly as well. Direct attempts to test this hypothesis have seldom been done but indirect evidence suggests that sperm may be coevolving with egg size (Raff *et al.* 1990; Levitan 1996; Levintan & Irvine 2001). Other evidence of rapid evolution comes from the phylogenetic analysis of sperm characteristics, which often suggests rampant secondary loss or reappearance of sperm structures (Rouse 1999; Koufopanou *et al.* 1999; Keys & Healy 2000). Further work is needed to clarify the role of sperm morphology in speciation and how quickly these traits change evolutionarily. This approach may make it possible to investigate the role of specific genes in the evolution of sperm morphology, and thereby elucidate some of the genetic mechanisms by which species evolve.

REFERENCES

Amy, R. L. 1983 Gamete sizes and developmental time tables of five tropical sea urchins. *Bull. Mar. Sci.* **33**, 173–176.

Arakaki, Y. 2000 The black *Echinometra* distributed in the Indo-West Pacific: a species complex. In *Echinoderms 2000* (ed. M. F. Barker), pp. 407–412. Lisse, The Netherlands: Swets & Zeitlinger.

Arakaki, Y. & Uehara, T. 1989 Comparative external morphology on the spermatozoa of four species, the family of Echinometridae, found on Okinawan reef flat. *Zool. Sci.* **6**, 1221.

Arakaki, Y. & Uehara, T. 1991 Physiological adaptations and reproduction of the four types of *Echinometra mathaei* (Blainville). In *Biology of Echinodermata* (ed. T. Yanagisawa, I. Yasumasu, C. Oguro, N. Suzuki & T. Motokawa), pp. 105–112. Rotterdam: Balkema.

Arakaki, Y. & Uehara, T. 1999 Morphological comparison of black *Echinometra* individuals among those in the Indo-West Pacific. *Zool. Sci.* **16**, 551–558.

Arakaki, Y., Uehara, T. & Fagoonee, I. F. 1998a Comparison between *Echinometra* species from Mauritius and Okinawa. In *Proc. 9th Int. Echinoderm Conf.* (ed. R. Mooi & M. Telford), pp. 547–551. Rotterdam: Balkema.

Arakaki, Y., Uehara, T. & Fagoonee, I. F. 1998b Comparative studies of the genus *Echinometra* from Okinawa and Mauritius. *Zool. Sci.* **15**, 159–168.

Aslan, L. M., Rahman, M. & Uehara, T. 1999 Relationship between egg size and larval size of two closely related species of sea urchins genus *Echinometra* and the egg size effects to the larval size of their reciprocal hybrids. *Biol. Mag. Okinawa* **37**, 63–73.

Barber, P. H., Palumbi, S. R., Erdmann, M. V. & Moosa, M. K. 2002 Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes, and consequences. *Mol. Ecol.* **11**, 659–674.

Bellwood, D. R. & Hughes, T. P. 2001 Regional-scale assembly rules and biodiversity of coral reefs. *Science* **292**, 1532–1534.

Briggs, J. C. 1999 Coincident biogeographic patterns: Indo–West Pacific Ocean. *Evolution* **52**, 326–335.

Chia, F. S., Atwood, D. & Crawford, B. 1975 Comparative morphology of echinoderm sperm and possible phylogenetic implications. *Am. Zool.* **15**, 533–565.

Civetta, A. & Singh, R. S. 1995 High divergence of reproductive tract proteins and their association with postzygotic reproductive isolation in *Drosophila melanogaster* and *Drosophila virilis* group species. *J. Mol. Evol.* **41**, 1085–1095.

Ferris, P. J., Pavlovic, C., Fabry, S. & Goodenough, U. W. 1997 Rapid evolution of sex-related genes in *Chlamydomonas*. *Proc. Natl Acad. Sci. USA* **94**, 8634–8639.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. C. 1994 DNA primers for amplification of mitochondrial cytochrome *c* oxydase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **3**, 284–299.

Geyer, L. B. & Palumbi, S. R. 2003 Reproductive character displacement and the genetics of gamete recognition in tropical sea urchins. *Evolution* **57**, 1049–1060.

Gomendio, M. & Roldan, E. R. S. 1991 Sperm competition influence sperm size in mammals. *Proc. R. Soc. Lond.* B **243**, 181–185.

Joly, D., Bressac, C., Devaux, J. & Lachaise, D. 1991 Sperm length diversity in Drosophilidae. *Dros. Inf. Serv.* **70**, 104–108.

Kelso, D. 1970 A comparative morphological and ecological study of two species of sea urchins: genus *Echinometra* in Hawaii. PhD thesis, University of Hawaii, Honolulu, USA.

Keys, J. L. & Healy, J. M. 2000 Relevance of sperm ultrastructure to the classification of giant clams (Mollusca, Cardioidea, Cardiidae, Tridacninae). In *The evolutionary biology of the Bivalvia* (ed. E. M. Harper, J. D. Taylor & J. A. Crame), pp. 191–205. The Geological Society of London.

Knowlton, N. 1993 Sibling species in the sea. *A. Rev. Ecol. Syst.* **24**, 189–216.

Koufopanou, V., Reid, D. G., Ridgway, S. A. & Thomas, R. H. 1999 A molecular phylogeny of the patellid limpets (Gastropoda: Patellidae) and its implications for the origins of their antitropical distribution. *Mol. Phylogenet. Evol.* **11**, 138–156.

Kumar, S., Tamura, K., Jakobsen, I. & Nei, M. 2001 MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.

Lamare, M. D. & Stewart, B. G. 1998 Mass spawning by the sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea) in a New Zealand fiord. *Mar. Biol.* **132**, 135–140.

LaMunyon, C. W. & Ward, S. 2002 Evolution of larger sperm in response to experimentally increased sperm competition in *Caenorhabditis elegans*. *Proc. R. Soc. Lond.* B **269**, 1125–1128. (DOI 10.1098/rspb.2002.1996.)

Lessios, H. A., Kessing, B. D. & Pearse, J. 2001 Population structure and speciation in tropical seas: global phylogeography of the sea urchin *Diadema*. *Evolution* **55**, 955–975.

Levitan, D. R. 1996 Effects of gamete traits on fertilization in the sea and the evolution of sexual dimorphism. *Nature* **382**, 153–155.

Levitan, D. R. & Irvine, S. D. 2001 Fertilization selection on egg and jelly-coat size in the sand dollar *Dendraster excentricus*. *Evolution* **55**, 2479–2483.

McCartney, M. A., Keller, G. & Lessios, H. A. 2000 Dispersal barriers in tropical oceans and speciation in Atlantic and eastern Pacific sea urchins of the genus *Echinometra*. *Mol. Ecol.* **9**, 1391–1400.

McMillan, W. O., Weigt, L. A. & Palumbi, S. R. 1999 Color pattern evolution, assortative mating, and genetic differentiation in brightly colored butterfly fishes (Chaetodontidae). *Evolution* **53**, 247–260.

Matsuoka, N. & Hatanaka, T. 1991 Molecular evidence for the existence of four sibling species within the sea-urchin, *Echinometra mathaei* in Japanese waters and their evolutionary relationships. *Zool. Sci.* **8**, 121–134.

Mayfield, J. A., Fiebig, A., Johnstone, S. E. & Preuss, D. 2001 Gene families from the *Arabidopsis thaliana* pollen coat proteome. *Science* **292**, 2482–2485.

Mead, K. S. 1996 Sex in the surf zone: the effect of hydrodynamic shear stress on the fertilization and early development of free-spawning invertebrates. PhD thesis, Stanford University, CA, USA.

Metz, E. C. & Palumbi, S. R. 1996 Positive selection and sequence rearrangements generate extensive polymorphism in the gamete recognition protein bindin. *Mol. Biol. Evol.* **13**, 397–406.

Metz, E. C., Kane, R. E., Yanagimachi, H. & Palumbi, S. R. 1994 Fertilization between closely related sea urchins is blocked by incompatibilities during sperm–egg attachment and early stages of fusion. *Biol. Bull.* **187**, 23–34.

Mita, M., Uehara, T. & Nakamura, M. 2002 Comparative studies on the energy metabolism in spermatozoa of four closely related species of sea urchins (genus *Echinometra*) in Okinawa. *Zool. Sci.* **19**, 419–427.

Mortensen, T. H. 1943 *Monograph of the Echinoidea: Camarondonta*. Copenhagen: C. A. Reitzel.

Nishihira, M., Sato, Y., Arakaki, Y. & Tsuchiya, M. 1991 Ecological distribution and habitat preference of four types of the sea urchin *Echinometra mathaei* on the Okinawan coral reefs. In *Biology of Echinodermata* (ed. T. Yanagisawa, I. Yasumasu, C. Oguro, N. Suzuki & T. Motokawa), pp. 91–104. Rotterdam: Balkema Press.

Palumbi, S. R. 1992 Marine speciation on a small planet. *Trends Ecol. Evol.* **7**, 114–117.

Palumbi, S. R. 1996 What can molecular genetics contribute to marine biogeography? An urchin's tale. *J. Exp. Mar. Biol. Ecol.* **203**, 75–92.

Palumbi, S. R. 1997 Molecular biogeography of the Pacific. *Coral Reefs* **16**, S47–S52.

Palumbi, S. R. 1999 All males are not created equal: fertility differences depend on gamete recognition polymorphisms in sea urchins. *Proc. Natl Acad. Sci. USA* **96**, 12 632–12 637.

Palumbi, S. R. & Metz, E. C. 1991 Strong reproductive isolation between closely related tropical sea urchins (genus *Echinometra*). *Mol. Biol. Evol.* **8**, 227–239.

Palumbi, S. R., Grabowsky, G., Duda, T., Geyer, L. & Tachino, N. 1997 Speciation and population genetic structure in tropical Pacific sea urchins. *Evolution* **51**, 1506–1517.

Palumbi, S. R., Cipriano, F. & Hare, M. P. 2001 Predicting nuclear gene coalescence from mitochondrial data: the three-times rule. *Evolution* **55**, 859–868.

Panhuis, T. M., Butlin, R., Zuk, M. & Tregenza, T. 2001 Sexual selection and speciation. *Trends Ecol. Evol.* **10**, 228–231.

Radwan, J. 1996 Intraspecific variation in sperm competition success in the bulb mite: a role for sperm size. *Proc. R. Soc. Lond.* B **263**, 855–859.

Raff, R. A., Herlands, L., Morris, V. B. & Healy, J. 1990 Evolutionary modification of echinoid sperm correlates with developmental mode. *Dev. Growth Differ.* **32**, 283–291.

Rahman, M., Uehara, T. & Pearse, J. 2001 Hybrids of two closely related tropical sea urchins (genus *Echinometra*): evidence against postzygotic isolating mechanisms. *Biol. Bull.* **200**, 97–106.

Rouse, G. W. 1999 Polychaete sperm: phylogenetic and functional considerations. *Hydrobiologia* **402**, 215–224.

Shapiro, A. M. & Porter, A. H. 1989 The lock-and-key hypothesis: evolutionary and biosystematic interpretation of insect genitalia. *A. Rev. Entomol.* **34**, 231–245.

Springer, V. G. & Williams, J. T. 1990 Widely distributed Pacific plate endemics and lowered sea-level. *Bull. Mar. Sci.* **47**, 631–640.

Swofford, D. 2001 *PAUP*: phylogenetic analysis using parsimony and other methods, v. 4.* Sunderland, MA: Sinauer.

Uehara, T., Shingaki, M. & Taira, K. 1986 Taxonomic studies in the sea urchin, genus *Echinometra*, from Okinawa and Hawaii. *Zool. Sci.* **3**, 1114.

Uehara, T., Asakura, H. & Arakaki, Y. 1990 Fertilization blockage and hybridization among species of sea urchins. In *Advances in invertebrate reproduction*, vol. 5 (ed. M. Hoshi & O. Yamashita), pp. 305–310. Amsterdam: Elsevier Science.

Uehara, T., Tsukahara, J. & Tsukashima, M. 1996 Distribution and characteristics of sea urchin genus *Echinometra* from Palau. Kagoshima University Research Centre South Pacific Occasional Papers, pp. 55–59.

Vacquier, V. D. & Moy, G. W. 1977 Isolation of bindin: the protein responsible for adhesion of sperm to sea urchin eggs. *Proc. Natl Acad. Sci. USA* **74**, 2456–2460.

Wyckoff, G. J., Wang, W. & Wu, C. I. 2000 Rapid evolution of male reproductive genes in the descent of man. *Nature* **403**, 304–309.