

Biogeography

A marine Wallace's line?

As most coral reef organisms with a pelagic larval phase are presumed to be readily dispersed between distant populations, sea-surface current patterns should be crucial for predicting ecological and genetic connections among threatened reef populations<sup>1</sup>. Here we investigate this idea by examining variations in the genetic structuring of populations of the mantis shrimp *Haptosquilla pulchella* taken from 11 reef systems in Indonesia, in which a series of 36 protected areas<sup>2</sup> are presumed to be connected by strong ocean currents. Our results reveal instead that there is a strong regional genetic differentiation that mirrors the separation of ocean basins during the Pleistocene low-sea-level stands, indicating that ecological connections are rare across distances as short as 300–400 km and that biogeographic history also influences contemporary connectivity between reef ecosystems.

Strong currents in Indonesian waters (Fig. 1a; up to  $1\text{ m s}^{-1}$ )<sup>3</sup> should facilitate the dispersal of marine larvae and are thought to be responsible for the invisibility of Wallace's line, the sharp biogeographic break that separates terrestrial forms from eastern and western Indonesia, in marine communities. Drifters have traversed 1,500 km through the Celebes Sea and Makassar Strait in four weeks<sup>4</sup>, indicating that planktonic larvae may travel great distances, yielding high connectivity between distant populations. However, fish<sup>5,6</sup> and invertebrate<sup>7</sup> larvae in the Caribbean and Australia show a surprisingly small amount of movement, challenging the idea of a strict association between dispersal potential and realized movement between marine populations. We therefore measured the genetic structuring of populations of the stomatopod *H. pulchella*, a benthic reef crustacean with a 4–6-week planktonic larval period and a dispersal potential that is conservatively estimated at 600 km.

Surprisingly, our results indicate that there are sharp genetic breaks among oceanographic regions. Populations north and south of the Flores and Java Seas, as close as 300 km apart, are distinguished by a broad genetic break perpendicular to Wallace's line ( $F_{ST}=0.821$  in analysis of molecular variance,  $P<0.001$ ; ref. 8) (Fig. 1b). Additionally, northern populations are significantly differentiated ( $F_{ST}=0.184$ ,  $P<0.001$ ), with genetically distinct populations (Fig. 1b) in the Bay of Tomini and in the Celebes, Flores and South China Seas, contradicting the hypothesis of strong connectivity among localities.

The geographic distribution of geneti-

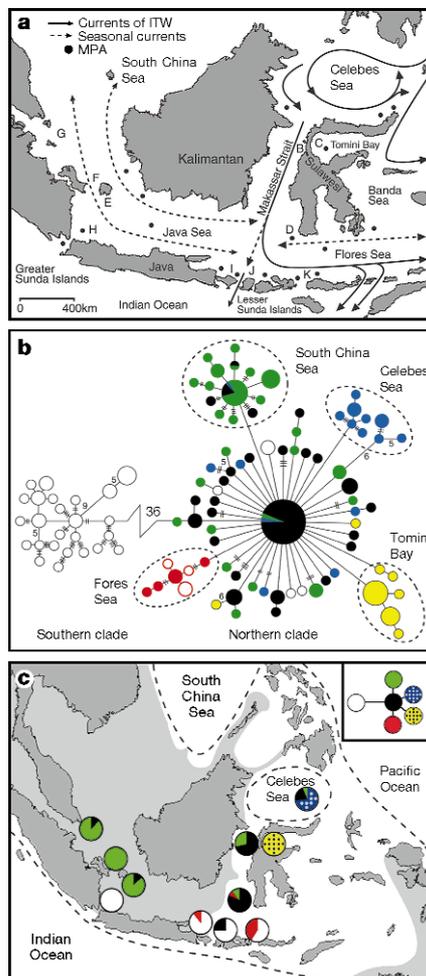
cally distinct populations reflects the greater isolation of ocean basins during Pleistocene low-sea-level stands<sup>9</sup>. The 'northern' and 'southern' clades may be relics of Indian and Pacific Ocean populations separated by the emergence of the Sunda and Sahul continental shelves during glacial maxima (Fig. 1c). Populations are also genetically distinct in the basins of the Celebes Sea, Tomini Bay and the South China Sea, which were partially enclosed during low-sea-level stands and have long been thought to be crucibles of species formation<sup>10,11</sup>.

If the observed genetic differentiation arose during periods of low sea level, it is surprising that dispersal following 6,000–10,000 years of modern oceanographic conditions has not erased these historical boundaries. Genetic breaks have been found between Indian and Pacific Ocean populations that are separated by thousands of kilometres<sup>12</sup>. However, the sharp genetic break described here that separates 'northern' and 'southern' populations a mere 300 km apart indicates the presence of a localized biogeographic boundary, suggesting the presence of a marine equivalent of Wallace's line. Our results indicate that predictions about connectivity based on generalized ocean

currents should also consider the biogeographic and oceanographic history.

Not all *H. pulchella* populations are genetically distinct, however. In the Spermonde archipelago (D in Fig. 1a), a collection of over 160 low coral islands in southwest Sulawesi, populations are genetically homogeneous over 10–100 km (5 populations,  $n=76$ ,  $F_{ST}=-0.03$ ,  $P=0.76$ ). These populations and the Pantaloan population (B in Fig. 1a) 400 km upstream are dominated by a single mitochondrial DNA haplotype (54% and 50%, respectively) that is the molecular ancestor of most northern haplotypes (Fig. 1b). Although dominant in the Spermonde and Pantaloan populations, this haplotype is also present at low frequency in populations from the South China Sea, the Celebes Sea and the Lesser Sunda Islands. Likewise, a haplotype common in Riau (G in Fig. 1a) and Belitung (E, F) occurs at low frequency in the Spermonde and Talau (A) (Fig. 1c). The distribution of these haplotypes indicates that gene flow is possible between distant populations, even if only rarely.

Information from other taxa should reveal whether these findings apply to other Indonesian reef species, but the association of stomatopod populations with old ocean



**Figure 1** Ocean currents and genetic structure of stomatopod populations. **a**, Dominant currents (solid lines) and seasonal currents (dashed lines) of the Indonesian throughflow (ITW)<sup>3</sup>. Filled circles, marine protected areas (MPA)<sup>2</sup>. Northern populations sampled were from regions marked A ( $n=20$ ), B ( $n=10$ ), C ( $n=21$ ), D (76 individuals from 5 different reef areas), E ( $n=10$ ), F ( $n=10$ ) and G ( $n=21$ ); southern populations were from regions H ( $n=14$ ), I ( $n=20$ ), J ( $n=5$ ) and K ( $n=6$ ). **b**, Unrooted minimum-spanning network (constructed using the program *Minispanet*; L. Excoffier) depicting the genetic relationship between 106 unique mitochondrial haplotypes from 213 individuals sampled from 11 regions throughout Indonesia. Haplotypes were identified by using the polymerase chain reaction to amplify 625 base pairs of the gene encoding cytochrome oxidase I (ref. 14) followed by automated sequencing on an ABI 377. The sizes of open circles (southern populations) and filled circles (northern populations) are proportional to haplotype frequencies and are separated by one mutational step, unless otherwise indicated by hatch marks or numerals. Dashed lines highlight clades found exclusively or primarily in one geographic region. Circles: red, Flores Sea; yellow, Tomini Bay; filled black, Makassar Strait; green, South China Sea; violet, Celebes Sea; white, Sunda Islands. **c**, Map of Indonesia at 18,000–20,000 years before present, when sea levels dropped by 130 m to expose the Sunda and Sahul continental shelves<sup>9</sup> (light grey). Dashed lines highlight distinct ocean basins formed during the lowering of sea levels. Pie diagrams indicate relative haplotype frequencies. Inset, simplification of **b**.

basins suggests that reef populations throughout Indonesia cannot simply be assumed to be interconnected units; marine reserves need to be designed<sup>1,13</sup> that also take biogeography and historical oceanography into account.

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Tokaimura accident

## Neutron dose estimates from 5-yen coins

After a criticality accident at a nuclear-fuel processing facility in Tokaimura, Japan, at 10:35 Japanese standard time on 30 September 1999, neutron emission continued until early the next morning. To estimate the neutron dose to the surrounding population, we collected 5-yen coins (37 weight per cent zinc) from places likely to have been exposed to radiation as a result of the accident and determined the amount of <sup>65</sup>Zn generated in them in the nuclear reaction <sup>64</sup>Zn + n → <sup>65</sup>Zn by spectrometrically measuring the photons emitted by <sup>65</sup>Zn. Our results indicate that people evacuated to 350 m outside the irradiated area still received a significant neutron dose.

The Japanese 5-yen coin is about 22 mm in diameter and 1.5 mm thick, weighs 3.75 g and has a central hole 5 mm wide. We chose this coin for monitoring neutron exposure because it is widely circulated, the zinc content is precisely controlled, and the <sup>65</sup>Zn generated has a convenient half-life (244.1 days) and  $\gamma$ -ray energy emission

Table 1 <sup>65</sup>Zn in 5-yen coins from houses around the accident site

Distance (m)	Direction from facility	<sup>65</sup> Zn concentration (Bq per kg Zn)	Statistical error (%)
100* ± 10	SSW	39.6	6.4
100* ± 10	SSW	37.0	6.4
100* ± 10	SSW	39.4	1.6
120 ± 12	W	17.4	14.0
135 ± 10	SSW	16.4	36.0
170 ± 10	SSW	9.0	32.0
210 ± 12	SSW	5.4	45.0
230 ± 12	SSW	4.0	22.7
250 ± 20	SSE	4.0	9.6
255 ± 20	WNW	3.8	35.0
320* ± 15	WSW	2.0	10.5
320* ± 15	WSW	1.9	23.0
360 ± 15	SE	1.5	24.1
380 ± 6	SE	1.4	51.2
460 ± 20	N	1.1	24.0
550* ± 12	SSE	0.86	18.4
550* ± 12	SSE	0.76	17.9

\*Samples that were separated and measured.

(1,115.5 keV). To obtain a record of the dosage of neutrons released as a result of the accident, we collected exposed coins from people's houses at distances 100–550 m from the facility.

Two types of detector were used to determine the concentration of <sup>65</sup>Zn in these coins: both are pure germanium detectors with a low background contribution, one of which (University of Tokyo) is covered with an inner copper shield 5 cm thick and a 5-cm layer of iron, with an outer lead casing of 10 cm — in this system, radon in the air is purged by using nitrogen gas; the other (Kyoto University) is covered with an inner copper shield of 10 cm and outer lead layer of 10 cm. Using these detectors, we tested 119 coins for a maximum of 1,000,000 seconds; some samples were measured in both systems to crosscheck for consistency. The scattering and self-absorption of 1,115.5-keV  $\gamma$ -rays in the coin samples were calculated by using the Monte Carlo method in three dimensions.

Table 1 shows <sup>65</sup>Zn concentrations measured in coins taken from houses in the neutron-affected areas around the nuclear-fuel processing facility. <sup>65</sup>Zn concentrations at distances ranging from 100 to 550 m from the plant dropped from 39.6 to 0.76 Bq per kg zinc, decreasing exponentially with increasing distance.

<sup>65</sup>Zn concentrations and neutron dosage at various distances from the accident site have also been estimated by considering the neutron energy spectrum in relation to distance (T. Imanaka, personal communication); values were calculated with the neutron transport programs DOT and

MORSE (ref. 1). Using these data, we were able to estimate approximate neutron doses from the <sup>65</sup>Zn concentrations shown in Table 1.

Ambient dose equivalent values (in millisieverts at 1 cm depth) were calculated as about 220 mSv at 100 m, 6 mSv at 350 m and 1.8 mSv at 550 m. These estimates agree well with the early official estimates<sup>2</sup>, which were revised to about half some weeks later<sup>3</sup>. Our estimated exposures indicate that those living 350 m away from the accident — the distance to which people were advised to evacuate — were also irradiated by neutron doses of over 1 mSv.

Our dose estimates include a bias because the neutron field in the environment might differ from that assumed in the calculation. If the neutron field is unimpeded, the <sup>65</sup>Zn in 5-yen coins can serve as an indicator of neutron dosage at various positions. Our results potentially offer information not only about the total neutron effect during the accident<sup>4</sup> but also about shielding by modern Japanese houses, given that the coins were recovered from indoors.

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