species that it shares with the IPR suggests that no location contributes as much to the overall alpha diversity of the Indian and Pacific oceans as does the IPR.

The distribution of biodiversity on Earth can be described in terms of a few well documented, and intriguing, small- to large-scale patterns. Our findings recognize a major link between the evolutionary processes regulating these patterns. That is, the processes of speciation, extinction and dispersal that yield large-scale patterns of species richness also seem to determine which species are present within local assemblages. Given the importance of the IPR to the overall structure of reef fish assemblages in the Indian and Pacific oceans, it should certainly be a target for strategic management and protection. □

Methods

Analyses were based on the presence or absence of 1,970 reef fish species in 70 locations in the Indian and Pacific oceans. These species belong to the families Labridae, Pomacentridae, Serranidae, Blenniidae, Apogonidae, Chaetodontidae, Acanthuridae, Scaridae, Holocentridae, Lutjanidae, Pomacanthidae, Scorpaenidae and Lethrinidae. These families are among the most diverse, best-known taxonomically, and represent >70% of the total species expected in any community. Owing to the high covariation in species richness among families[8], these families are a good indicator for the remaining diversity of species. The database includes all data from the 63 locations used in ref. 8. Data for the following locations were added: the Philippines, Madagascar, Easter Island, Cook Islands (all from www.fishbase.org), Cocos Keeling[21]; Gorgona Reef[22]; and Korea (http://ricos.cnu.ac.kr/~kocofish/list/elistintro.htm). Data for the following locations were updated: Galapagos[23]; Gulf of California[24]; and Malpelo (C.M., personal observations). All species records were corrected for synonymy and other taxonomic problems using The Catalog of Fishes (http://www.calacademy.org/research/ichthyology/catalog/fishcatsearch.html). More than 300 species were duplicated in the original database as a result of synonymy, misspelled names or misallocation of species to families.

Pelagic larval durations were obtained for 95 labrid species and 116 pomacentrids. These species represent 28% of all labrid species and 42% of the pomacentrids. Data were obtained from references 19, 25–27.

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Mechanism of genetic exchange in American trypanosomes

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The kinetoplastid Protozoa are responsible for devastating diseases[1]. In the Americas, *Trypanosoma cruzi* is the agent of Chagas' disease—a widespread disease transmissible from animals to humans (zoonosis)—which is transmitted by exposure to infected faeces of blood-sucking triatomine bugs[2]. The presence of genetic exchange in *T. cruzi* and in *Leishmania* is much debated[3,4]. Here, by producing hybrid clones, we show that *T. cruzi* has an extant capacity for genetic exchange. The mechanism is unusual and distinct from that proposed for the African trypanosome, *Trypanosoma brucei*[5]. Two biological clones[6] of *T. cruzi* were transfected to carry different drug-resistance markers[7,8], and were passaged together through the entire life cycle. Six double-drug-resistant progeny clones, recovered from the mammalian stage of the life cycle, show fusion of parental genotypes, loss of alleles, homologous recombination, and uniparental inheritance of kinetoplast maxicircle DNA. There are strong genetic parallels between these experimental hybrids and the genotypes among natural isolates of *T. cruzi*. In this instance, aneuploidy through nuclear hybridization results in recombination across far greater genetic distances than mendelian genetic exchange. This mechanism also parallels genome duplication[9,10].

The species *T. cruzi* is divided into two divisions (I and II) on the basis of isoenzyme phenotypes, DNA profiles, ribosomal and mini-exon DNA sequence polymorphisms, and microsatellite analysis[11–13]. In addition, *T. cruzi* II, which predominates where Chagas' disease is more severe[14], can be divided into up to five sublineages (IIa–e)[11,15]. Population genetics has emphasized clonal propagation and the lack of genetic exchange in *T. cruzi* when natural isolates, from dispersed geographical localities, have been tested for random mendelian genetic exchange[4,8,16]. Nevertheless, recent phylogenetic evidence suggests that *T. cruzi* IId and IIe may have an ancient hybrid origin[3,16]. Surprisingly, meticulous quantification studies demonstrated that

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biological clones of a single *T. cruzi* strain had between 30% and 70% more DNA than a parental stock[17], indicating some rapid genetic mechanism for radical change in DNA content. Our experiments reconcile these observations by providing evidence of genetic hybridization in *T. cruzi*, and through evidence that this leads to aneuploidy observed in natural populations.

Two primary isolates of *T. cruzi* I were obtained from an undisturbed enzootic cycle of transmission at a site in the Brazilian Amazon basin, where putative parental and hybrid phenotypes were circulating sympatrically[6]. A pair of putative parental biological clones, P1 and P2, were transfected, respectively, with a modified form of pTEX carrying the hygromycin-resistance gene (M.C.T., unpublished observations) and with pTEX[18], which carries the neomycin (G418)-resistance gene. *Trypanosoma cruzi* P1-*hyg* and *T. cruzi* P2-*neo* were then passaged singly or together through the various life cycle stages (see Methods). Immediately after co-passage, recovered organisms were placed in culture with hygromycin and G418 to select for populations resistant to both drugs. Double-drug pressure was sustained throughout biological cloning of the hybrids and all their subsequent growth stages.

Fifty Vero cell cultures infected with a mixture of P1-*hyg* and P2-*neo* yielded one population of organisms resistant to both hygromycin and G418. Six derived biological clones (1C2, 1D12, 2A2, 2C1, 2D9, 2F9) were resistant to both drugs. No double-drug-resistant populations were recovered from mixed axenic epimastigote cultures, from mixed passage through triatomine bugs, or from mixed passage in SCID mice.

We used several methods to investigate the nature of the six double-drug-resistant *T. cruzi* clones. DNA amplification (Fig. 1a) showed that the six clones contained both the *hyg* and *neo* genes. Isoenzyme analysis (Fig. 1b) and karyotype analysis (Fig. 1c) showed that the clones shared parental characters and were at least, in part, hybrids. Random amplification of polymorphic DNA (RAPD) with three of seven primers revealed characteristic sharing of bands between progeny clones and parents; controls with non-transformed P1 and P2 showed that shared bands were not attributable to the episomes (Fig. 1d). Microscopy of Giemsa-stained preparations demonstrated that the hybrid clones were not binucleate.

P1-*hyg* and P2-*neo* were screened for microsatellite repeat-length polymorphisms. For six informative loci, which distinguished P1-*hyg* from P2-*neo*, each of the six progeny clones showed all parental alleles (Fig. 1e; see also Supplementary appendix 1a, b). For four other loci, with one homozygous and one heterozygous parent, the unique allele of the heterozygous parent was passed to all progeny. However, for locus L660, the allele of the homozygous parent (P2-*neo*) was absent from all progeny, indicating allele loss (Supplementary appendix 1a).

Allele loss was also apparent among progeny at the nuclear tryparedoxin locus (*tpn*1)[19], in that none of the progeny showed the unique *tpn*1 allele of the heterozygous parent P2-*neo* at position 172 base pairs (bp) (A/T) and 204 bp (A/C), with P1-*hyg* being homozygous at both positions (T and C respectively). Sequence analysis of the nuclear genes glucosephosphate isomerase (*gpi*), an intergenic region (*tcp*)[20] and phosphoglucomutase (*pgm*) indicated fusion and revealed evidence of recombination. The parents, P1 and P2, at the *gpi*, *tcp* and *pgm* loci had a combined total of three, six and four genotypes, respectively (Supplementary appendix 2). As evidence of fusion, one progeny clone (2A2) had all but one of these genotypes. Five clones had a full complement of both parental genotypes at one or more of the three loci, but several parental genotypes were not recovered from the hybrids. Putative recombination was present at the *gpi* locus (5′ 600 bp), the *tcp* intergenic region (5′ 400 bp) and the *pgm* locus (380 bp) in the form of mosaic parental genotypes within amplified DNA clones derived from the biological clones of the six double-drug-resistant progeny (Methods; see also Supplementary appendix 2 (GenBank accession numbers AY227811–AY227891)). Recovery of six mosaics identifiable by different genetic markers was not compatible with slippage and polymerase chain reaction (PCR) artefacts, although the frequency of recombination events could not be ascertained.

The mitochondrial maxicircle sequence revealed a difference in the NADH dehydrogenase subunit 1 (*nd1*) gene between P1-*hyg* (A) and P2-*neo* (G) at position 102 bp (613 bp in the amplicon sequenced). Five progeny (1C2, 2A2, 2C1, 2D9, 2F9) inherited the P1-*hyg* genotype and one (1D12) inherited the P2-*neo* genotype; none of the progeny inherited both types. Parental cytochrome oxidase II sequences were identical.

Analogy between the experimental hybrids and natural populations was shown by finding evidence for three or more size-length polymorphisms at single microsatellite loci within biological clones for one field isolate of *T. cruzi* I and four field isolates of *T. cruzi* II (Supplementary appendix 3). The *T. cruzi* I (TCI) isolate showed polyploidy at half of the microsatellite loci examined (6 out of 12). Multiple microsatellite genotypes previously thought to indicate multiclonality[13] may be explicable predominantly by polyploidy. The CL Brener strain also diverges from uniform diploidy, with triploid sections of the genome (genome project strain, J. Kelly,

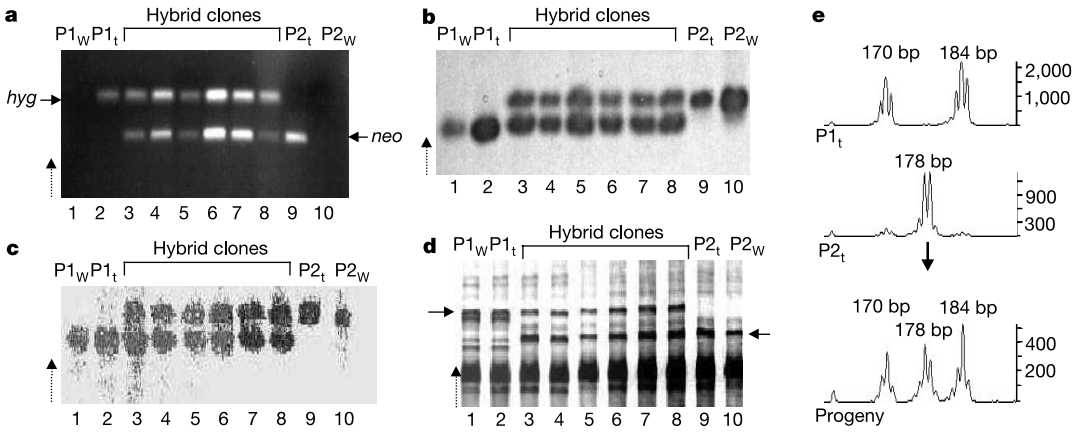

Figure 1 Hybrids of parental phenotypes and genotypes in experimentally derived double-drug-resistant biological clones of *T. cruzi*. **a**, Ethidium-stained agarose gel of multiplex polymerase chain reaction (PCR) to detect *hyg* and *neo*. Lanes (for **a**–**d**) are: *T. cruzi* P1 ($P1_W$: lane 1); transformed P1-*hyg* ($P1_t$: lane 2); six double-drug-resistant hybrids (lanes 3–8); transformed *T. cruzi* P2-*neo* ($P2_t$: lane 9); and wild-type *T. cruzi* P2 ($P2_W$: lane 10). **b**, Starch gel electrophoresis showing PGM phenotypes. **c**, Southern blot of contour-clamped homogeneous electric field (CHEF) gel probed to show cysteine protease karyotypes. **d**, One of three random amplification of polymorphic DNA (RAPD) profiles (smaller products omitted) showing shared bands (arrowed). **e**, One (A427) of six polyploid microsatellite loci (y = fluorescent units).

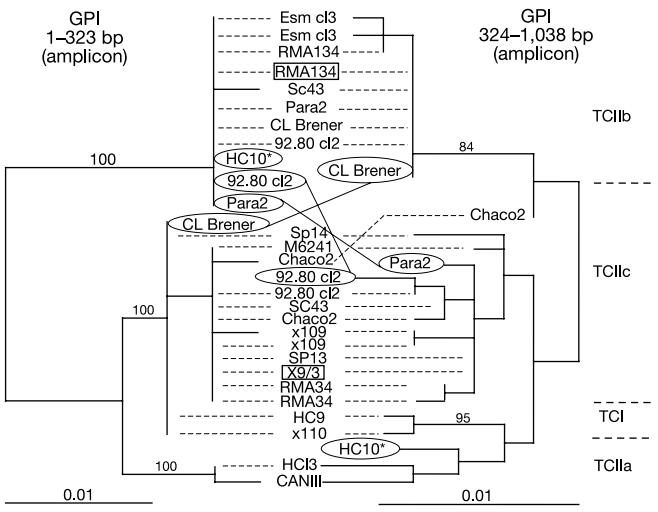

Figure 2 Phylogenetic support in GPI demonstrated by the incongruence between phylogenies for *T. cruzi* IIb (TCIIb; plus d and e) and TCIIc (plus d and e) lineages for putative recombinants, where a circled isolate indicates a putative recombinant and a boxed isolate indicates that parents were used for maximum likelihood breakpoint analysis. Asterisks indicate that the breakpoint cannot be illustrated conveniently. Bootstrap values are provided above the lines.

personal communication). The apparent uniparental inheritance of maxicircle DNA in the hybrid clones is compatible with the universally haploid genotypes at the same locus in 46 reference strains[3].

Phylogenetic analyses gave unequivocal evidence of genetic recombination among natural populations. Thus gene mosaics were seen in 7 out of 18 isolates, over 1,700 bp of total nucleotide sequence, with mosaics between TCIIb and TCIIc genotypes (six isolates) and between TCIIb and TCI (one isolate) identified using both bootstrap methods (Fig. 2; see also Supplementary appendix 4) and bootscan analysis (Fig. 3). All sites of recombination were confirmed using a maximum likelihood test of recombination breakpoints ($P \leq 0.01$ in all cases). Description of subspecific groups by phylogenetics showed that genotypes from TCIId and TCIIe were split between groups TCIIb and TCIIc. Trees were congruent with previous phylogenetic studies (Supplementary appendix 5). There was supporting evidence of genetic recombination for the *gpi* and *tcp* loci in at least 39% of all reference strains or field isolates.

Recovery of hybrids from mammalian cells implies that genetic exchange can occur in vertebrate reservoir hosts of *T. cruzi*. Life-long *T. cruzi* infection in mammalian hosts[2], subject to multiple infections and chemotherapy, offers prolonged exposure to selection of hybrid genotypes. In contrast, genetic exchange in *T. brucei*, which has important epidemiological consequences, takes place in the tsetse fly and probably involves meiosis[4,5,21]. *Trypanosoma cruzi* is considered to be quite distinct from *T. brucei* (separated by ≥100 million years (Myr))[3]; for example, *T. cruzi* invades cells and replicates intracellularly in the vertebrate host, whereas *T. brucei* is entirely extracellular. Aneuploidy through hybridization explains the wide range in DNA content of *T. cruzi*[17], and reconciles the paradox between apparent clonal propagation despite the occurrence of recombination, because a deviation from mendelian heterozygosity would result from either clonality or aneuploidy and subsequent allele loss: determination of the frequency of recombination requires different genetic models and predictions. Absence of detectable panmixia in bacteria is observed even when frequent recombination is occurring[22] and high levels of mitotic recombination through gene conversion are known; for example, in oomycetes[23]. Most notably, aneuploidy via nuclear hybridization is a non-mendelian mechanism that may lead to marked speciation

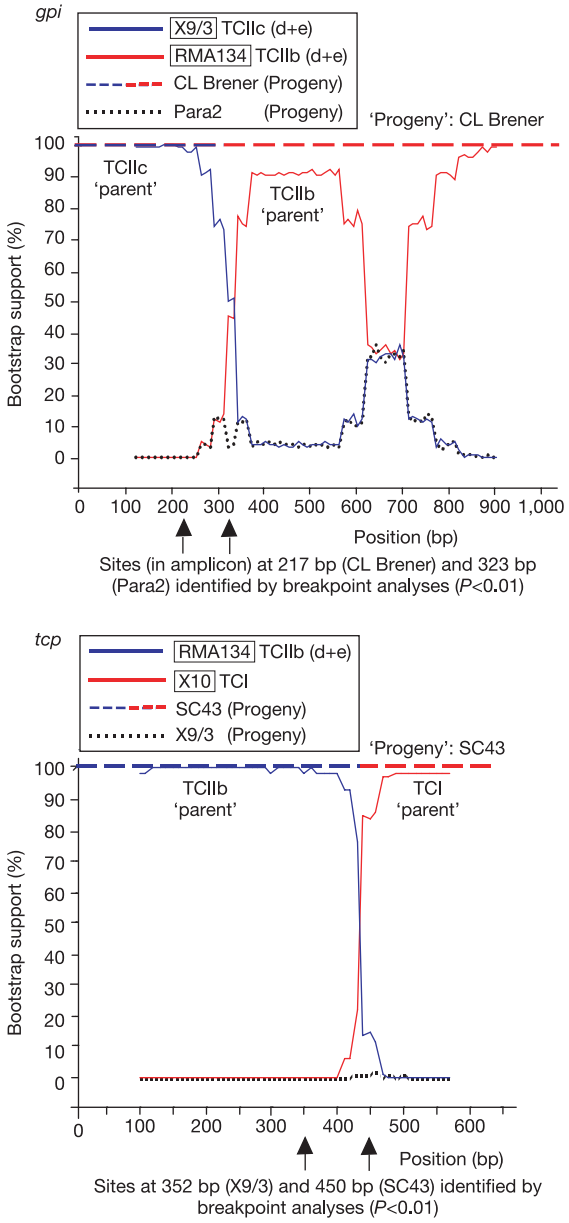

Figure 3 Phylogenetic support for mosaic gene, or split gene, structures in putative recombinant 'progeny' between parental TCIIb and TCIIc (*gpi* locus), and parental TCIIc and TCI (*tcp* locus) using bootscan analysis. Several additional examples of recombination between TCIIb and TCIIc were observed for *tcp* (Supplementary appendix 4b). All putative sites of recombination, or breakpoints, are significant using a maximum likelihood test ($P < 0.01$).

events; it is also likely to be present in *Leishmania*[24]. The minimal divergence time separating TCIIb from the TCIIc and TCI sister lineages is estimated to be 2–9 Myr (ref. 3) using nuclear loci (8–20 Myr for a mitochondrial maxicircle locus). Thus the observed homologous recombination between these lineages spans a vast temporal divergence, based on conservative calibration points[25]. This phenomenon provides an added dimension to the concept of genome duplication, in that it may also operate in phylogenetic groups close to the origins of eukaryotes[9,10]. □

Methods

Selection of double-drug-resistant clones

Biological clones of *T. cruzi* were prepared by microscopic selection of single cells and culture. The following *in vitro* or *in vivo* systems[26] were used for co-passage of *T. cruzi* P1-*hyg* and P2-*neo*. First, P1-*hyg* and P2-*neo* epimastigotes were mixed in equal amounts,

grown axenically *in vitro* using supplemented RPMI 1640 medium, and at intervals after the stationary phase (21 days) they were passaged into new cultures containing each drug or both drugs (hygromycin, 150 $\mu g\,ml^{-1}$; G418, 120 $\mu g\,ml^{-1}$). Second, mammalian cell (Vero) monolayers were infected with P1-*hyg* and P2-*neo* using stationary-phase, mixed axenic cultures containing epimastigotes and metacyclic trypomastigotes. Trypomastigotes from pseudocysts were recovered periodically between days 7 and 28, and grown as epimastigotes in axenic culture for drug sensitivity tests. Third, triatomine bugs were membrane fed on mouse blood containing P1-*hyg* and P2-*neo* trypomastigotes derived from Vero cell monolayers. Bugs were dissected 25–30 days later, *T. cruzi* was re-isolated by culture on biphasic blood agar, and was passaged into axenic culture to obtain sufficient organisms for testing drug sensitivities. Last, groups of three immunocompromised (SCID) mice were inoculated with a mixture of faeces from triatomine bugs carrying P1-*hyg* or P2-*neo*. Populations were subsequently retrieved from infected mice into axenic culture and placed under drug pressure.

Determination of phenotype and genotype

DNA purification was carried out by phenol/chloroform extraction and ethanol precipitation or using DNeasy (Qiagen). Amplification reactions used the following conditions: denaturation for 5 min at 94 °C, then 30 cycles of 94 °C (1 min), 50–62 °C (1 min; depending on the primer T_m) and 72 °C (1 min per 1,000 bp), followed by 10 min at 72 °C. Primers are described in Supplementary appendix 6. PGM phenotype determination and RAPD analysis were as described previously[6]. Episomes were detected by multiplex amplification using primers designed to the hygromycin phosphotransferase and neomycin phosphotransferase genes. For karyotype analysis we used a Bio-Rad CHEF Mapper with an autoalgorithm for separation of 0.4–2.2-Mb fragments, followed by Southern blotting and hybridization with radiolabelled probes.

The following DNA sequences were amplified, some with *Taq* Extender; Stratagene: (1) *tpn1*[19] (tryparedoxin: GenBank accession number AF106855; 435 bp); (2) *gpi* (1,038 bp); (3) putative *pgm* (380 bp) (TIGR database (http://www.tigr.org) accession number TC1375), identified by similarity between human and putative *Leishmania pgm*; (4) *tcp*, an intergenic region (760 bp, including gaps) flanked by 3′ *tcp17* and partial 5′ *tcpgp2* (ref. 20); (5) a mitochondrial locus (1,078 bp) spanning the maxicircle-encoded genes cytochrome oxidase subunit II (*coII*) and *nd1* (ref. 3); and (6) *dhfr-ts* (1,042 bp)[3]. PCR products from all loci were cloned into pGEM T (Promega), except where no heterozygous alleles were detectable (mitochondrial DNA[3] of progeny; *gpi* of some isolates). For each isolate a minimum of three (*gpi*) and either three or six (*tcp*) clones were sequenced on a capillary sequencer (Beckman) or an ABI 377 using relevant kits.

Genotypes were also determined at eight microsatellite loci[13], with primers labelled with FAM, NED and HEX, and sized against the ROX 500 marker (ABI) using an ABI 377. An additional 12 microsatellite loci were identified by searching the *T. cruzi* genome database for dinucleotide repeats (TIGR database; see also Supplementary appendix 7). Genescan and Genotyper software (Applied Biosystems) were used to automate measurements of allele length. All microsatellite loci were amplified from P1-*hyg* and P2-*neo* and their double-drug-resistant progeny using standard conditions; a subset (MCLE01, MCLEF10, MCLEG10, SCLE10 and SCLE11 (ref. 13), and A427, A831.3, E801, J356 and N060 (Supplementary appendix 7)) was amplified from all reference strains and field isolates (Supplementary appendix 8).

Phylogenetic analysis

Nucleotide sequences were aligned using Clustal X[27] then edited by hand, and are available on request. Primarily, sites of recombination were examined by bootscan analysis[28] using the Kimura two-parameter model. Recombination sites were investigated further by maximum likelihood breakpoint analysis[29]. All maximum likelihood parameter estimates comprising a four-category gamma distribution and a transition/transversion ratio (κ) were obtained with the tree bisection reconnection (TBR) heuristic search (where reconnection limit = 4) using PAUP* 4.0b (D. L. Swofford). Each data set of field isolates and reference strains was also subject to refined split decomposition analysis (Kimura three-parameter model)[30].

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Water transport in plants obeys Murray's law

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The optimal water transport system in plants should maximize hydraulic conductance (which is proportional to photosynthesis[1–5]) for a given investment in transport tissue. To investigate how this optimum may be achieved, we have performed computer simulations of the hydraulic conductance of a branched transport system. Here we show that the optimum network is not achieved by the commonly assumed pipe model of plant form[6–8], or its antecedent, da Vinci's rule[9,10]. In these representations, the number and area of xylem conduits is constant at