Opinion

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The evolution of *Trypanosoma cruzi*: the 'bat seeding' hypothesis

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Recent discussions on the evolution of *Trypanosoma cruzi* have been dominated by the southern super-continent hypothesis, whereby *T. cruzi* and related parasites evolved in isolation in the mammals of South America, Antarctica and Australia. Here, we consider recent molecular evidence suggesting that *T. cruzi* evolved from within a broader clade of bat trypanosomes, and that bat trypanosomes have successfully made the switch into other mammalian hosts in both the New and Old Worlds. Accordingly, we propose an alternative hypothesis – the bat seeding hypothesis – whereby lineages of bat trypanosomes have switched into terrestrial mammals, thereby seeding the terrestrial lineages within the clade. One key implication of this finding is that *T. cruzi* may have evolved considerably more recently than previously envisaged.

Trypanosoma cruzi: Chagas disease, hosts, transmission and evolution

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, continues to rank among the most important public health problems in South America and despite extensive research efforts focusing on both the parasite and the vector over more than 100 years [1], the evolutionary history of this parasite remains to be resolved. *T. cruzi sensu lato* (s.l.) comprises *Trypanosoma cruzi cruzi*, the causative agent of Chagas disease in humans, and *Trypanosoma cruzi marinkellei*, which is found only in South American bats. In addition to humans, the hosts of *T. c. cruzi* include a range of wild animals, including many species of marsupials and xenarthrans (armadillos, sloths, anteaters), a variety of rodents, primates, carnivores, bats and some wild pigs, and a variety of domesticated animals, including dogs, cats, pigs and possibly goats [2]. The parasite is generally transmitted between mammalian hosts by contamination with the feces of infected triatomine bugs and ingestion of infected triatomines by mammals. In addition, vertical transmission between mother and infant via breast feeding or blood contamination, transmission via blood transfusion and mechanical transmission between host animals, for example via anal glands in opossums, have been documented and may be more or less important depending on local circumstances.

Since the late 1990s, discussion on the evolution of *T. cruzi* trypanosomes has been dominated by the southern super-continent hypothesis [3–5] which suggested that *T. c. cruzi* emerged from trypanosomes that had been isolated in terrestrial mammals of the New World since the break-up of the continental landmass that included Australia, Antarctica and South America approximately 40 million years ago (Figure 1). Although this hypothesis is widely accepted, here we highlight several recent studies that suggest that *T. c. cruzi* was originally a bat parasite that subsequently made the switch into terrestrial mammals.

The southern super-continent hypothesis and its implications

The first comprehensive phylogenetic analyses of disease causing trypanosomes demonstrated that *T. cruzi* was distantly related to another human infective species, *Trypanosoma brucei* s.l., the causative agent of African sleeping sickness, and that both appear to have evolved human parasitism independently [5,6]. Stevens *et al.* [3–5,7] explained the distribution of taxa in 18S rDNA phylogenies using known patterns of host biogeography and geological evidence.

The divergence of African tsetse-transmitted trypanosomes (the Salivaria, also known as the *Trypanosoma brucei* clade) was dated to around 100 mybp, when Africa became isolated from the other continents (Figure 1) [5,7]. The clade consisted predominantly of trypanosomes from African mammals, whereas species from African amphibia (*Trypanosoma mega*) and reptiles (*Trypanosoma grayi*, *Trypanosoma varani*) were unrelated. At this time (100 mybp) the ancestors of many extant mammalian groups were present, but had not begun major diversification [8].

Separation of *T. cruzi* and related trypanosomes from African trypanosomes was similarly dated; the composition of the *T. cruzi* clade at the time included two trypanosomes from South American terrestrial mammals' *T. cruzi* and *Trypanosoma rangeli*, and a trypanosome from an Australian kangaroo [5,9]. This distribution reflects the present day distribution of marsupial mammals; the split of South America from Antarctica and Australia is thought to have occurred later than the separation from Africa [8] (Figure 1). Three bat trypanosomes were also in the *T. cruzi* clade [3]: *T. cruzi marinkellei* from South American bats, and *Trypanosoma dionisii* and *Trypanosoma vespertilionis* that are

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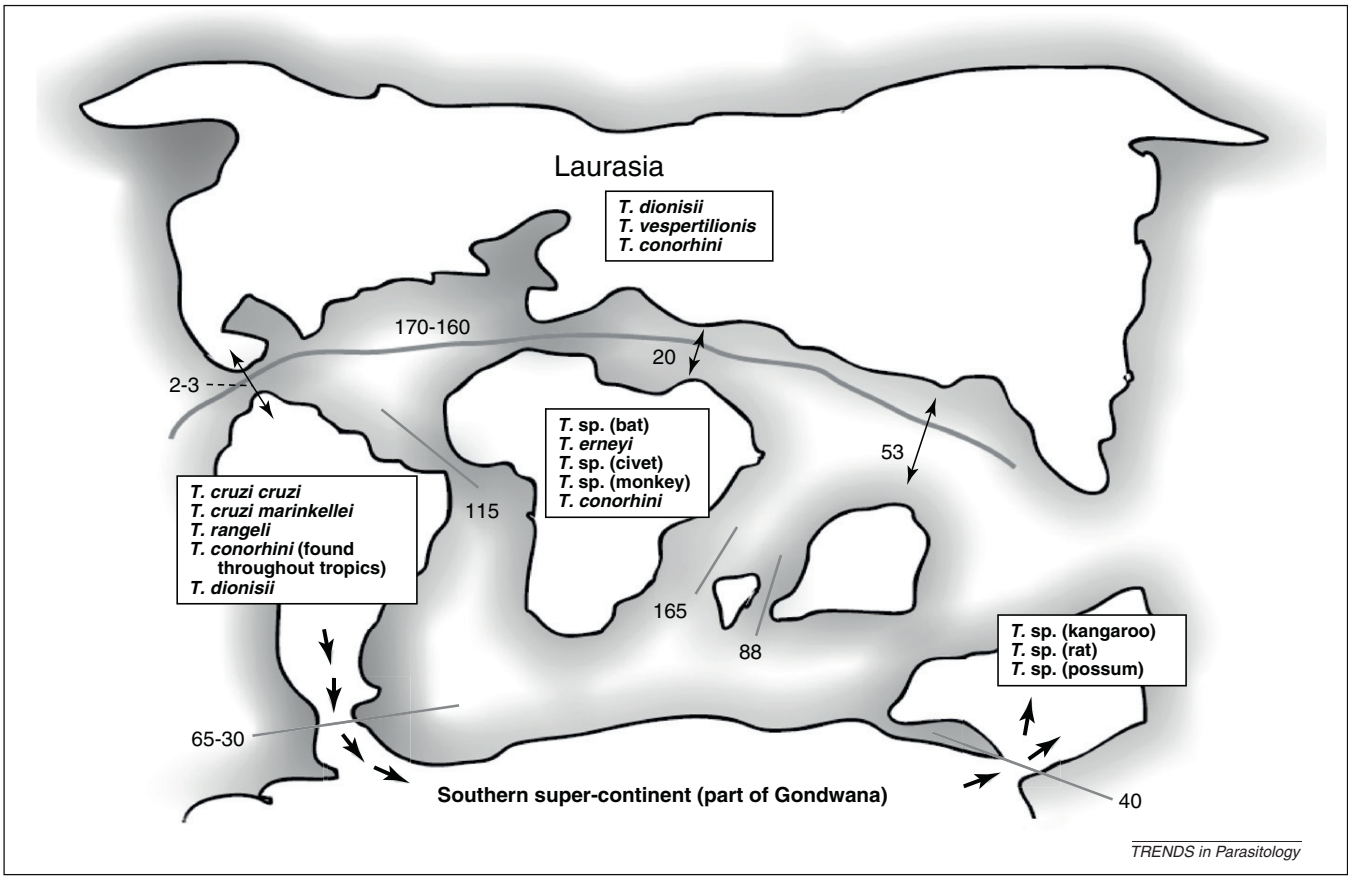

Figure 1. Current geographical distribution of *T. cruzi* clade trypanosomes. Current distribution of trypanosome taxa (characterized by molecular methods) in the *T. cruzi* clade, mapped onto the positions of continents in the late cretaceous; adapted from Cox and Moore [8]. Gray lines show dates of splits between landmasses. Double-headed arrows show dates of collision between continents. Single-headed arrows show the direction of spread of marsupials to Australia from South America via Antarctica [8]. All numbers are millions of years ago. Africa, Antarctica, Australia, India, Madagascar and South America formed the southern super-continent of Gondwana.

present in the Old World. The latter two species were hypothesized to have originated in the New World, and dispersed to the Old World by infected bats flying between the continental landmasses. Latterly, the clade was expanded to include *Trypanosoma conorhini*, a rat trypanosome found throughout the tropics, including South America. At this time, this species was hypothesized to have originated in the New World and spread subsequently to the Old World via rats in ships [10].

Thus, the southern super-continent hypothesis, that is, the idea that *T. cruzi* and its relatives originated when South America, Australia and Antarctica were linked in a single continent – and that at this time marsupials were the only mammals present – has been used to explain these patterns and host distributions, and has dominated discussion of *T. cruzi* evolution for the past decade. For example, it has been used for calibration of molecular trees in a range of studies [7,11,12]. Recently, Flores-López and Machado [13] used 32 genes to date the divergence of extant lineages of *T. cruzi* to within the past 3 million years, with trees calibrated using a divergence date of 100 million years for the split between *T. cruzi* and *T. brucei*. Likewise, Lewis *et al.* [14] used the same date to estimate the emergence of the two hybrid lineages of *T. c. cruzi* that are associated with severe clinical disease to within the past 60 000 years.

New evidence and the southern super-continent hypothesis

New evidence has emerged in recent years that has challenged some of the assumptions of the super-continent hypothesis.

Low diversity of T. cruzi *clade in South American terrestrial mammals*

A wide diversity of trypanosome species within the subgenus *Schizotrypanum* has been described from South American mammals using bloodstream morphology, although it has long been suspected that some of these species were synonyms of *T. c. cruzi* [15]. An increasing number of studies have now characterized trypanosomes from a wide range of South American terrestrial mammals using molecular techniques, revealing high diversity within both *T. c. cruzi* and *T. rangeli*. To date, however, no further *bona fide* species in the *T. cruzi* clade have been discovered in terrestrial mammals from this continent. This apparent low level of diversity does not accord with what might be expected if *T. cruzi*-group trypanosomes had been present in the continent for 40 million years. For example, some parasite cultures identified as *Trypanosoma leeuwenhoeki* (from a sloth, *Choloepus hoffmanni*) and *Trypanosoma minasense* (from a neotropical primate, the squirrel monkey, *Saimiri boliviensis*) on the basis of morphology have

turned out to be *T. rangeli* in 18S rDNA phylogenies [10]. Other recently described parasites from the region have fallen outside the *T. cruzi* clade, for example, isolates morphologically similar to *T. minasense*, which were sequenced directly from blood samples from primates imported into Japan from South America [16] and a trypanosome from a dog [17]. Arguably, further species related to *T. cruzi* have not been detected because many of these studies have first cultured the trypanosomes [18,19], which may have inadvertently screened out species less well suited to growth in culture. For example, trypanosomes with morphology similar to that of subgenus *Megatrypanum* are often encountered in the blood of American primates, bats and didelphids, but cannot be isolated using standard media. The apparently low level of diversity so far detected in Australian *T. cruzi* clade trypanosomes is discussed in Box 1.

The origin of *T. conorhini* is far from clear, but new evidence indicates an Old World origin for this species. Crucially, new phylogenetic studies suggest that its vector, the triatomine bug *Triatoma rubrofasciata*, may have originated in the Old World; it is most closely related to species of the Asiatic triatomine genus *Linshcosteus* [20,21]. This cosmopolitan rat trypanosome has only been reported in domestic rats in Brazil and its vector is strongly associated with rats. Its history and origins are thus probably linked to those of its hosts and vector, both of which now appear to be in the Old World (the brown rat, *Rattus norvegicus*, and the black rat, *Rattus rattus*, are thought to have originated in Asia rather than in South America and were dispersed there by ships [22]).

Thus, based on current evidence, *T. c. cruzi* and *T. rangeli* are the only trypanosomes in the *T. cruzi* clade that we can be confident were in South American terrestrial mammals before the arrival of humans in the New World. By contrast, over the past decade the known diversity of trypanosomes infecting African tsetse flies has been shown to be considerably higher than previously thought [23,24]. This finding supports the hypothesis that the group (African trypanosomes) has been present in the continent for a considerable period of time [5]; although, as has been shown previously [7], rates of evolution can vary considerably between (and within) the trypanosome clades. Thus, time estimates derived from mutation rates should always be treated with appropriate caution and are not directly comparable between African trypanosomes and the *T. cruzi* group.

T. cruzi *clade in African terrestrial mammals*

The second challenge to the southern super-continent hypothesis comes from a study in which a large range of vertebrates from Cameroon in West Africa were surveyed for the presence of pathogenic African trypanosomes [25]. The species-specific primers used in the study left some trypanosomes unidentified and later phylogenetic analysis placed two trypanosomes from a palm civet and a monkey in the *T. cruzi* clade [26]. The monkey trypanosome was most closely related to a European bat trypanosome, *T. vespertilionis* (Figure 2), whereas the civet trypanosome appeared to be more closely related to *T. conorhini*.

Thus, *T. cruzi* clade parasites are present in terrestrial mammals from the New World, Australia and Africa, questioning the role of geographical isolation in restricting movement of these trypanosomes (Box 1). It is also possible that other *T. cruzi* clade trypanosomes are yet to be discovered elsewhere in the Old World. This possibility has been explored by Weinman *et al.* [27], who found that some monkey trypanosomes from South East Asia developed

Box 1. Geographical isolation and trypanosomatid evolution

Vicariance biogeography has been used to explain the current day distribution of many groups of organisms [8]. The approach uses information on plate tectonic movements and the break-up of continents due to continental drift to explain extant species' distribution patterns. Several studies have used vicariance biogeography to explain the distribution of trypanosomatid taxa. Lake *et al.* [42] used the break-up of Africa and South America to date the divergence of *Leishmania* and *Trypanosoma*, whereas Fernandes *et al.* [43] dated the split between Old and New World *Leishmania* to the separation of the continental landmasses.

Several recent studies question the importance of geographical isolation in determining patterns of trypanosomatid biogeography. The position of the kangaroo trypanosome at the periphery of the *T. cruzi* clade was crucial in forming the southern super-continent hypothesis [5]. At the time, an unrelated trypanosome from a wombat was the only other trypanosome from an Australian marsupial included in phylogenies [5]. The diversity of marsupials in Australia largely results from the isolation of the continent 40 million years ago, when it became separated from Antarctica (Figure 1), which allowed diversification of its marsupial fauna. Rodents and bats are the only placental mammals to have entered and successfully colonized Australia before human introductions. Yet, although molecular surveys in the past decade have revealed considerable diversity in trypanosomes of Australian mammals, such studies have not revealed a deep sister clade in Australia as might be expected if trypanosomes within the *T. cruzi* clade had been present within the continent for 40 million years. Trypanosomes from a native Australian rodent, *Rattus fuscipes* [44], and a possum, *Trichosurus vulpecula* [45], appear to be relatively close relatives of the kangaroo trypanosome. However, other newly discovered trypanosome lineages from Australian marsupials are more closely related to trypanosomes outside of Australia than to each other. Examples include a koala trypanosome, whose closest known relative is a trypanosome from an American bird [46]. *Trypanosoma copemani* and related trypanosomes from wombats, quokkas, potoroos, woylies and ticks from Australia [47,48] have relatives in Japanese ticks [49], Eurasian badgers [5,50] and South American dogs [17]; a wallaby trypanosome is related to trypanosomes found in terrestrial leeches (from Australia and Asian forests) and a primate trypanosome, *Trypanosoma cyclops*, from Southeast Asia [51]. Thus, although the mammals of Australia have been geographically isolated, their trypanosomes have not, casting doubt on the original significance of the kangaroo trypanosome in defining a southern super-continent hypothesis [5].

This pattern mirrors the findings from studies of some other trypanosomatid groups. Within some genera of monogenetic (single host) trypanosomatids, some closely related monoxenous insect parasites have been described from China and the Neotropics, suggesting recent dispersal across substantial distances and biogeographical barriers [52]. Similarly, a recently discovered *Leishmania* taxon from an Australian kangaroo [53,54] appears closely related to a rodent species, *Leishmania enriettii*, a species found infecting rodents in Brazil and another from an AIDS patient in Martinique (West Indies) [55]. Most recently, strains of *T. dionisii* have been discovered in Europe that are very closely related to those in the New World, suggesting recent and probably natural movement, further demonstrating the ability of bat trypanosomes to disperse between geographically separate continents [31].

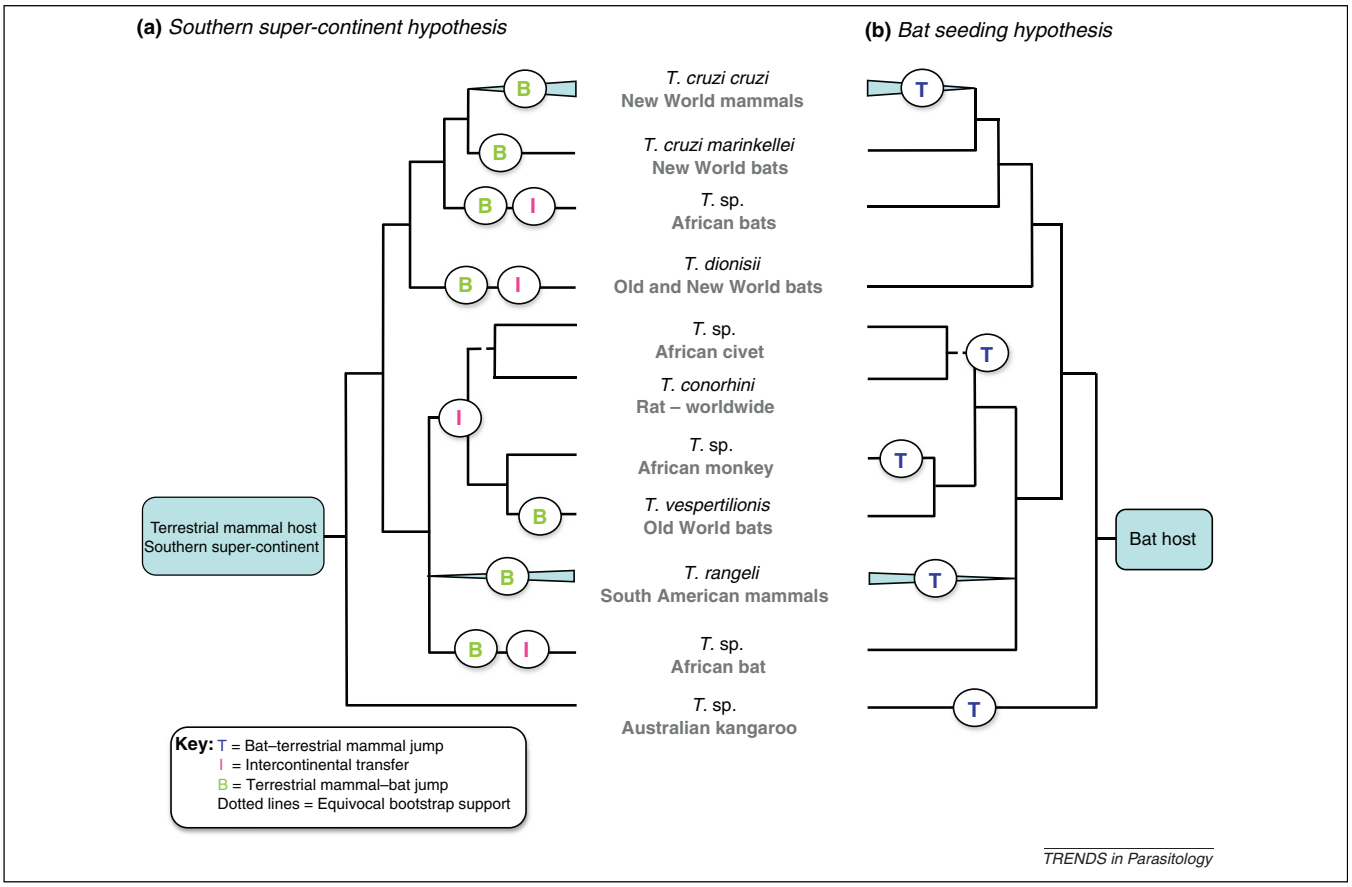

Figure 2. Two hypotheses for the evolution of the *T. cruzi* clade. Relationships within the *T. cruzi* clade, showing two hypotheses for the evolution of the *T. cruzi* clade. **(a)** The southern super-continent hypothesis and **(b)** the bat seeding hypothesis. The two scenarios presented represent (in our opinion) the simplest explanations of the relationships that exist between these taxa. Isolates from Brazilian bats with isoenzyme and RAPD profiles distinct from *T. cruzi* and *T. rangeli*, but more closely related to *T. vespertilionis* and *Trypanosoma hastatus* [40], have been described as a new species *Trypanosoma desterrensis* [41]; however, comparison of the spliced-leader sequences indicates that they are highly similar to Brazilian isolates of *T. dionisii*, which was not compared by these authors (M.M.G. Teixeira *et al.*, unpublished). There is also some doubt as to whether this *T. vespertilionis* is the originally described species in North Africa in 1905 and in several European countries in 1904–1907 [15]. *T.* (*Schizotrypanum*) *vespertilionis* was described in Brazil in 1936 and it has never been clearly ascertained whether descriptions from the New and Old Worlds relate to the same species. To date, moreover, *T. vespertilionis* matching the Old World isolate shown in this figure has not been described from Brazil with molecular methods, whereas molecular techniques have identified *T. dionisii*, which has not been previously reported in South America (M.M.G. Teixeira *et al.*, unpublished); such findings suggest that *T. vespertilionis* as originally described in the subgenus *Schizotrypanum* and *T. dionisii* may be synonyms.

readily in triatomine bugs, whereas others did not. Hoare [15] suggested that the species that developed in triatomines were *T. conorhini*. A refractory species, *Trypanosoma cyclops*, was later found to be more closely related to *Trypanosoma theileri* species than to *T. cruzi* [28], although, to date, no molecular work has been undertaken on *bona fide* isolates of Weinman's unclassified triatomine-infective trypanosome from Indonesian macaques. In addition, uncharacterized trypanosomes from slow loris (*Nycticebus coucang*) from Malaysia were detected in muscle tissue cells [29], a trait associated only with trypanosomes in the subgenus *Schizotrypanum*.

Phylogenetic positioning of bat trypanosomes

Recent phylogenetic studies have included a greater diversity of bat trypanosomes and have resolved relationships within the *T. cruzi* clade using concatenated gene sequences [18,26,30–32]. The most parsimonious explanation for the relationships observed within the group is that the *T. cruzi* clade was primitively a bat clade, which has made multiple jumps into terrestrial mammals, rather than it being a terrestrial clade with multiple lineages jumping into bats. Thus, it now appears that *T. c. cruzi* evolved from a bat trypanosome (Figure 2), because its closest known living relative is *T. c. marinkellei* (from South American bats), followed by *Trypanosoma erneyi*, a recently described species from bats in Mozambique, Africa [30], and *T. dionisii*, from both Old and New World bats [5,10,18,31,32]. Although a bat origin of the clade requires a minimum of five independent switches from bats to terrestrial mammals, this is – on the basis of currently available taxa – a more parsimonious explanation than a South American terrestrial origin for the clade, which requires six or seven independent switches from terrestrial mammals to bats and four independent movements of the group from the Old World to the New World (Figure 2). As yet, no trypanosomes outside of the *T. cruzi* clade (except *Trypanosoma evansi* in vampire bats [15]) are known to have made the switch into bats, even though bats presumably feed on the vectors of various other trypanosomes, which may indicate that the *T. cruzi* clade trypanosomes have some ancestral adaptations to bat parasitism. Additional surveys will probably further increase our knowledge of the diversity of bat trypanosomes within

the clade, because, although the studies of the past decade have already demonstrated increased diversity, many species of bat remain to be been sampled and several geographical regions, such as Africa, Asia and Oceania, are at this time largely unsampled (note that Hoare [15] includes several descriptions of trypanosomes in the blood of bats from these regions).

Both *T. c. cruzi* [18,33–36] and *T. rangeli* [34] have been detected in South American bats, although the prevalence of *T. c. cruzi* in bats is very low compared to the prevalence of both *T. c. marinkellei* and *T. dionisii*. In addition, one genotype of *T. c. cruzi*, Tcbat, has so far only been found in bats, but its host range may well include other arboreal and terrestrial mammals because it has been shown to infect mice under experimental conditions [33]. Interestingly, Tcbat is most closely related to *T. c. cruzi* TcI, a group that is preferentially associated with didelphids and *Rhodnius* spp. in arboreal niches [2]. Likewise, the *T. rangeli* lineage TrE has only been found in bats and the triatomine *Rhodnius pictipes* from Central and Amazon regions in Brazil, and is probably not restricted to bats, because it was also found in experimentally infected mice [34]. As yet, it is unclear whether these taxa are representative of ancestral lineages or lineages that have been recently derived from terrestrial lineages by, e.g. predation by bats on infected triatomine bugs that share their roosts, such as caves, tree holes, palms and other sylvatic refugia.

The bat seeding hypothesis

The various strands of evidence that have emerged in the past decade do not support the southern super-continent hypothesis and instead can be brought together to form a new hypothesis – the bat seeding hypothesis – a scenario outlined in Figure 2. In this scenario, the common ancestor of trypanosomes in the *T. cruzi* clade (at least the common ancestor of *T. rangeli* and *T. cruzi*) was a bat trypanosome. These bat trypanosomes then diversified and became geographically widespread. Various trypanosomes within this group independently switched from bats into terrestrial mammals, with one such switch giving rise to *T. c. cruzi*. Bat–terrestrial mammal switches occurred at least five times (Figure 2), a number that is likely to increase as the true diversity of trypanosomes in bats and terrestrial mammals becomes known. Such switches would and may continue to be facilitated by the sharing of niches of bats, terrestrial mammals and invertebrate vectors, where a range of arthropod genera will feed on both bats and terrestrial mammals. Establishment of transmission cycles involving terrestrial mammals would have been facilitated by 'host fitting', whereby parasites adapt more readily to novel hosts that are related to their current host [32]. For instance, the only known vectors of *T. c. marinkellei* are triatomines of the genus *Cavernicola*, which are found associated with bat colonies in caverns, hollow trees and palms [37,38]; we envisage that adaptation of the parasite to a vector that feeds on bats may have facilitated transition to other species of triatomines that feed on arboreal and terrestrial mammals.

On the basis of the evidence now available, the *T. cruzi* clade of trypanosomes are likely to have diversified sometime after bats underwent major diversification, approximately 70–58 million years ago [39] and, moreover, could have done so considerably more recently. The relatively high genetic diversity within *T. c. cruzi*, its wide geographical distribution in the New World and its wide host range (both in mammals and triatomines) point to a pre-human origin of this species. Thus, humans would have first been exposed to *T. cruzi* when they migrated to the New World approximately 30 000 years ago and first came into contact with the arthropod vectors of the parasite. Subsequently, infection would have become prevalent when homes became infested with triatomine species as a natural extension of their preferred habitat.

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