

# Extreme inbreeding in *Leishmania braziliensis*

Virginie Rougeron<sup>a,1</sup>, Thierry De Meeûs<sup>a,b</sup>, Mallorie Hide<sup>a</sup>, Etienne Waleckx<sup>a</sup>, Herman Bermudez<sup>c</sup>, Jorge Arevalo<sup>d</sup>, Alejandro Llanos-Cuentas<sup>d</sup>, Jean-Claude Dujardin<sup>e</sup>, Simone De Doncker<sup>e</sup>, Dominique Le Ray<sup>e</sup>, Francisco J. Ayala<sup>f,1</sup>, and Anne-Laure Bañuls<sup>a</sup>

<sup>a</sup>Génétique et Evolution des Maladies Infectieuses, IRD/CNRS/UMI (UMR 2724), Montpellier F-34394, France; <sup>b</sup>Laboratoire de Recherches et de Coordination sur les Trypanosomoses TA A-17/G, UMR IRD-CIRAD 177, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France; <sup>c</sup>Faculty of Medicine, Universidad Mayor San Simon, P.O. Box 4866, Cochabamba, Bolivia; <sup>d</sup>Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 460, Lima, Peru; <sup>e</sup>Unit of Molecular Parasitology, Institute of Tropical Medicine, B-2000 Antwerp, Belgium; and <sup>f</sup>Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697-2525

Contributed by Francisco J. Ayala, April 21, 2009 (sent for review January 23, 2009)

*Leishmania* species of the subgenus *Viannia* and especially *Leishmania braziliensis* are responsible for a large proportion of New World leishmaniasis cases. The reproductive mode of *Leishmania* species has often been assumed to be predominantly clonal, but remains unsettled. We have investigated the genetic polymorphism at 12 microsatellite loci on 124 human strains of *Leishmania braziliensis* from 2 countries, Peru and Bolivia. There is substantial genetic diversity, with an average of  $12.4 \pm 4.4$  alleles per locus. There is linkage disequilibrium at a genome-wide scale, as well as a substantial heterozygote deficit (more than 50% the expected value from Hardy–Weinberg equilibrium), which indicates high levels of inbreeding. These observations are inconsistent with a strictly clonal model of reproduction, which implies excess heterozygosity. Moreover, there is large genetic heterogeneity between populations within countries (Wahlund effect), which evinces a strong population structure at a microgeographic scale. Our findings are compatible with the existence of population foci at a microgeographic scale, where clonality alternates with sexuality of an endogamic nature, with possible occasional recombination events between individuals of different genotypes. These findings provide key clues on the ecology and transmission patterns of *Leishmania* parasites.

clonality | microsatellites | population genetics | endogamy | heterozygote deficiency

Leishmaniasis are worldwide vector-borne diseases of humans and domestic animals, caused by protozoan parasites of the genus *Leishmania*. These parasitoses are a serious public health problem, with about 350 million persons at risk and 2,357,000 new cases per year (1). Leishmaniasis occur on all continents except Antarctica. There are more than 20 described species causing human infections (review in ref. 2). Clinical symptoms range from asymptomatic, cutaneous, and mucocutaneous to visceral forms, depending on the *Leishmania* species. Visceral leishmaniasis is mainly caused by species from the *Leishmania donovani* complex; cutaneous and mucocutaneous forms are associated with species from the *Viannia* and *Leishmania* subgenera (3–5). *L. braziliensis* causes cutaneous and mucocutaneous leishmaniasis in South America, where these are a severe public health problem.

Despite numerous studies and recent advances in the molecular genetics of these organisms, the reproductive mode of these parasites remains unsettled. Tibayrenc and Ayala (6) proposed that all (or most) *Leishmania* species are clonal. Other authors have challenged this hypothesis, based on pulse field gel electrophoresis (PFGE) data, and argued that some *Leishmania* species are potentially automictic, with frequent genetic exchanges (7). Several studies suggest that recombination may occur in *Leishmania*, and that other complexities may exist (see review in ref. 2). For example, based on evidence from PFGE analyses, Bañuls et al. (8) have proposed the occurrence of pseudorecombination in *Leishmania* populations. Moreover, several genetic studies indicate genetic recombination between *Leishmania* individuals, despite lack of evidence for a sexual stage (9–16). In any case, the molecular data

suggest that, after a hybridization event, hybrids propagate clonally in natural populations (9, 12).

The prevailing hypothesis is that *Leishmania* displays a clonal mode of reproduction with occasional pseudorecombination and intragenic recombination, which mimic sexual reproduction processes, and that infrequent genetic exchanges take place in wild populations. Nevertheless, much remains to be elucidated as this interpretation is challenged by certain data, such as the absence of large excess in heterozygosity, as expected in clonal diploids (17, 18), and the lack of a clear structure in individualized lineages at the intraspecific level (2). Indeed, in a clonal model, an excess of heterozygotes and significant linkage disequilibrium are expected. Thus, the known results have failed to resolve the issue of clonality vs. sexuality in these protozoan parasites. Improved knowledge of the population structure and reproductive strategy of *Leishmania* parasites would provide a better understanding of their transmission patterns, as well as useful information for diagnostic purposes, epidemiological surveys, and drug and vaccine development.

Microsatellite loci are highly polymorphic, codominant, abundant throughout the genome, and relatively easy to assay (19, 20). In *Leishmania*, microsatellite studies are relatively recent; a small number of polymorphic microsatellites have been described for *Leishmania* species of the *Viannia* subgenus and especially for *L. braziliensis* (21). We analyze the population structure of *L. braziliensis* in several natural populations from South America (Peru and Bolivia), based on 12 microsatellite loci previously described (22). Peru and Bolivia are 2 of 7 world countries that report 90% of cutaneous leishmaniasis cases. Our population genetics analysis may be the first study of this kind for this *Leishmania* species. It reveals an unexpectedly high level of inbreeding within local samples, a large part of which is explained by local heterogeneity (Wahlund effect), probably due to a microgeographic population substructure, but also to the occurrence of mixed-mating events that include a significant contribution of endogamy (i.e., recombination between 2 genetically identical cells).

## Results

We analyzed 124 human strains of *L. braziliensis* from 4 samples: 2 from the Pilcopata department in Peru, isolated in either 1993 or 1994, and 2 from Chapare Natural Park in Bolivia, isolated in either 1994 or 1998 (Tables 1 and 2). Both sites are located in the Amazonian forest and extend over large areas of great faunal and floral diversity.

Author contributions: V.R., T.D.M., and A.-L.B. designed research; V.R., M.H., E.W., H.B., J.A., A.L.-C., S.D.D., D.L.R., and A.-L.B. performed research; V.R., T.D.M., M.H., and A.-L.B. analyzed data; and V.R., T.D.M., M.H., J.-C.D., F.J.A., and A.-L.B. wrote the paper.

The authors declare no conflict of interest.

<sup>1</sup>To whom correspondence may be addressed. E-mail: fjayala@uci.edu or rougeron.virginie@gmail.com.

This article contains supporting information online at [www.pnas.org/cgi/content/full/0904420106/DCSupplemental](http://www.pnas.org/cgi/content/full/0904420106/DCSupplemental).



**Table 2. Data set with each sample code, the country, and the year of collection and all genotypes obtained at each locus by PCR**

Sample code	Country	Year	Loci											
			AC01	AC16	AC52	ARP	ITSbraz	Ibh3	LRC	CAK	EMI	LBA	G09	E11
LC1568	Peru	1993	202–202	149–161	104–104	139–139	102–102	130–130	124–124	162–162	165–165	180–180	150–152	102–102
LC2231	Peru	1994	202–202	149–155	084–098	139–145	106–106	130–130	132–132	162–162	189–189	178–178	150–154	096–096
LC2280	Peru	1994	198–210	151–161	084–084	137–139	104–106	130–130	132–132	158–158	191–191	176–182	150–162	098–102
LC2282	Peru	1994	206–212	149–149	100–100	151–153	102–102	128–128	120–126	162–162	165–165	176–180	156–156	096–096
LC2291	Peru	1994	202–202	151–151	120–120	133–149	102–106	130–130	128–128	162–162	175–185	174–182	148–148	100–100
LC2292	Peru	1994	204–210	149–149	084–120	139–139	104–104	130–130	132–132	160–160	189–189	174–180	148–152	096–096
LC2293	Peru	1994	202–202	149–151	098–098	133–149	102–106	130–130	128–128	164–168	175–185	174–174	150–152	100–102
LC2308	Peru	1994	204–210	151–169	100–100	149–149	100–102	116–130	120–128	160–160	185–185	174–180	154–154	098–100
LC2310	Peru	1994	204–210	159–159	084–094	137–139	102–102	130–130	132–132	160–160	189–189	176–182	148–156	100–100
LC2315	Peru	1994	202–210	151–161	108–108	141–141	102–102	130–130	130–134	162–162	189–189	176–182	154–154	100–104
LC2316	Peru	1994	200–204	147–161	088–088	135–135	100–100	118–130	130–138	160–160	185–185	176–180	148–154	098–098
LC2318	Peru	1994	202–202	149–167	092–108	139–139	102–102	130–130	130–130	162–162	177–195	174–182	152–152	100–100
LC2319	Peru	1994	200–200	149–157	104–110	141–147	104–108	130–130	124–124	164–164	193–193	174–184	152–152	100–100
LC2320	Peru	1994	202–210	149–155	118–118	155–155	104–104	128–128	132–132	162–162	189–189	174–182	150–154	102–102
LC2321	Peru	1994	208–208	149–149	086–106	149–155	100–100	128–128	122–130	162–162	189–189	176–176	148–156	098–106
LC2352	Peru	1994	202–212	149–161	110–110	143–143	102–104	130–130	126–130	162–162	191–191	178–180	150–154	098–098
LC2353	Peru	1994	202–212	149–161	084–094	129–139	102–102	128–128	132–132	158–162	191–191	176–180	150–152	098–098
LC2355	Peru	1994	210–210	149–159	098–122	129–137	102–102	128–128	132–132	158–160	189–189	176–180	150–150	100–100
LC2367	Peru	1994	202–202	149–161	084–098	131–139	102–106	130–130	132–132	158–164	191–191	174–180	148–148	098–098
LC2368	Peru	1994	202–202	149–161	084–098	135–141	102–104	130–130	132–132	158–162	191–191	174–180	148–148	100–100
LC2369	Peru	1994	200–210	149–161	096–096	135–141	102–106	130–130	126–132	158–164	189–189	174–182	150–150	098–098
LC2371	Peru	1994	200–200	151–161	084–098	135–141	100–106	130–130	132–132	158–164	189–189	174–180	148–148	098–098
LC2373	Peru	1994	202–202	151–161	084–098	133–139	102–104	130–130	132–132	162–162	191–191	174–180	150–150	100–100
LC2284	Peru	1994	210–210	151–161	086–116	139–139	102–102	130–130	130–130	158–160	189–189	174–180	150–152	100–102
LC2322	Peru	1994	202–202	149–161	094–110	149–149	104–104	130–130	130–130	160–160	191–193	174–182	154–154	100–102
CH12B	Bolivia	1994	204–206	149–159	126–126	155–157	102–102	116–130	124–130	168–168	183–183	174–180	154–154	100–100
CH15	Bolivia	1994	206–206	147–157	124–124	155–157	102–102	116–130	122–134	164–164	189–189	168–174	154–154	100–106
CH17	Bolivia	1994	202–202	147–161	096–096	131–131	102–102	130–130	132–132	164–164	185–191	168–168	154–154	098–102
CH25B	Bolivia	1994	202–204	149–149	100–100	121–121	102–102	130–130	124–134	158–164	179–187	168–172	152–168	100–102
CH29B	Bolivia	1994	204–206	149–159	126–126	125–157	102–102	128–128	130–134	158–166	183–185	174–180	154–154	100–100
CUM106	Bolivia	1994	204–206	147–159	100–100	129–131	102–102	128–128	124–128	164–164	185–189	174–174	152–154	100–100
CUM107	Bolivia	1994	202–202	149–149	092–092	121–121	106–106	120–120	118–120	156–156	189–189	170–170	144–144	090–090
CUM31	Bolivia	1994	206–206	149–159	126–126	135–137	102–102	116–128	122134	162–172	187189	168–174	154–154	102–108
CUM38	Bolivia	1994	202–204	149–149	098–104	129–135	102–102	130–130	114134	160–160	185–185	168–168	154–154	102–102
CUM41	Bolivia	1994	202–202	149–149	114–120	131–131	102–102	118–128	126–130	160–160	185187	168–172	154–154	100–102
CUM50	Bolivia	1994	206–206	149–159	100–110	129–147	102–102	116–128	122–124	164–164	185–187	174–174	154–154	100–102
CUM52	Bolivia	1994	206–206	149–149	100–110	129–147	102–102	116–128	122–124	164–164	185–187	174–174	154–154	100–102
CUM53	Bolivia	1994	206–206	149–149	112–112	129–147	104–104	116–116	122–132	164–170	185–189	174–174	154–154	098–104
CUM55	Bolivia	1994	206–206	149–149	126–126	137–137	102–102	128–130	114–130	158–170	187–189	162–168	154–154	098–102
CUM59	Bolivia	1994	206–206	149–159	124–124	129–129	102–102	136–136	122–134	162–174	187–189	168–174	154–154	102–108
CUM65	Bolivia	1994	202–202	149–159	114–114	129–137	102–102	116–128	122–130	164–164	179–185	174–174	154–154	102–102
CUM67	Bolivia	1994	208–208	149–149	088–108	149–155	102–106	128–128	122–130	160–166	187–193	176–178	150–156	100–108
CUM68	Bolivia	1994	202–206	151–151	128–128	129–129	102–108	128–128	122–130	164–168	187–189	168–174	152–154	102–102
CUM82	Bolivia	1994	202–206	149–149	098–112	131–131	100–102	136–136	124–132	164–164	179–189	180–180	154–154	098–100
CUM84	Bolivia	1994	206–206	149–149	096–096	131–131	100–100	116–130	130–130	158–166	181–189	174–174	152–152	100–100
CUM97	Bolivia	1994	206–206	149–159	100–110	129–147	102–102	116–128	122–124	164–164	185–187	174–174	154–154	100–102
CUM96	Bolivia	1994	204–204	149–161	084–098	129–137	102–102	128–128	130–130	162–172	191–193	168–182	154–154	100–102

Selection can strongly affect allele and genotypic frequencies. Underdominance, which decreases the fitness of heterozygous individuals, would result in deficiency of heterozygous genotypes relative to Hardy–Weinberg expectations. Underdominance, however, is not expected to be frequently encountered in nature because it is highly unstable (the rarest allele tends to disappear). We have observed similar  $F_{IS}$  patterns across all 12 (dinucleotide, noncoding) microsatellite loci. Widespread, almost genome-wide, underdominance would be required to fit our data, which does not seem reasonable.

Parasites of the Trypanosomatidae family, such as *Leishmania* species, are characterized by genetic plasticity, so that they can use different pathways to generate genetic diversity (e.g., gene conversion; refs. 39 and 40), a process of unidirectional transfer of genetic material between members of a multigenic family (41). Gene conversion generates a transition from the heterozygous stage to the homozygous stage, and thus can result in substantial heterozygote deficiency (40). Given that we obtained similar findings across the 12 independent microsatellite loci, gene conversion could account for our findings only if it occurred among all of the loci studied, across the entire genome, which seems unlikely. If gene

conversion significantly affected microsatellite loci heterozygosity, a negative relationship would be expected between differences in allele size in heterozygous individuals and the number of such heterozygous individuals in the data set. This is because heterozygotes recently aroused through mutation have less chance of being immediately converted again compared with older heterozygotes, and with microsatellite loci in clonal organisms, old heterozygotes are expected to carry the most distant alleles in terms of size (30, 31). If gene conversion occurs frequently, microsatellite loci should restore heterozygosity through mutation and thus between alleles that are close in length. We obtained a significant negative relative relationship between the size difference in bases between alleles ( $\Delta$ ) and the number of heterozygous individuals,  $N_{Hz}$ , only for locus *E11* (see Fig. S1 and SI Text). This locus is located 60 bp before the trifunctional enzyme alpha subunit mitochondrial precursor-like gene, and this observation might reflect frequent conversion in this genomic region. If we exclude *E11* from our data, the overall high  $F_{IS}$  values persist.

Clustering within each sample, as performed by BAPS, results in a substantial (40%) decrease in  $F_{IS}$  values. This is consistent with the existence of a strong Wahlund effect within each





tested by 10,000 randomizations of alleles within subpopulations (for  $F_{IS}$ ) and of individuals between subpopulations (for  $F_{ST}$ ). For  $F_{IS}$ , the statistic used was Weir and Cockerham's estimator  $f_i$ ; for  $F_{ST}$ , the statistic used was the log-likelihood ratio  $G$  (55) summed over all loci. Confidence intervals were estimated by bootstrapping over loci or jackknifing over populations with FSTAT. From the  $F_{IS}$  parameter, a potential selfing rate  $s$  was inferred using the formula  $s = (2 * F_{IS}) / (1 + F_{IS})$  (e.g., ref. 29).

Linkage disequilibrium between pairs of loci (nonrandom association of alleles at different loci) was assessed with a randomization test (genotypes at 2 loci are associated at random a number of times). The statistic used was the log likelihood ratio  $G$  summed over all subpopulations. Because this procedure was repeated on all pairs of loci, we applied the sequential Bonferroni correction (56) to the  $P$  values ( $P$  value  $\times$  number of tests).

The #3.2 software identifies a hidden structure within populations through a Bayesian analysis. It clusters individuals into genetically distinguishable groups based on allele frequencies. This software was used to detect possible Wahlund effects and has been successfully applied to other parasites (16, 57). The BAPS software used stochastic optimization to infer the posterior mode of the genetic structure. To obtain the best distribution of the 4 populations under study, we ran the program many times to obtain the number of clusters. We also checked that nonstructured populations would not give the same results as ours. This was done by running BAPS on populations simulated with EASYPOP (version 2.0.1). Each of the 4 samples was submitted to a clustering exploration by BAPS with 160 runs

with a maximum number of clusters set to 20.  $F_{IS}$  was recalculated in each best distribution identified by BAPS and compared  $F_{IS,C}$  with the initial  $F_{IS}$  using a unilateral Wilcoxon signed-rank test for paired data, the pairing units being the 12 loci. If  $F_{IS,C}$  is lower than  $F_{IS}$ , it is probable that the initial subsamples were composed of several genetically distinct entities (e.g., geographical microstructure or subpopulations).

To estimate the contribution of macrogeography (between Bolivia and Peru) corrected for the effect of the subpopulation structure (between BAPS clusters), we used HIERFSTAT (version 0.03–2) software (58). This test uses the same statistics as those used for  $F_{ST}$  analyses, but the permutation procedure takes into account the hierarchy of the population structure. Differentiation between clusters within countries,  $F_{Cluster/Country}$ , is tested by randomization of individuals between clusters of the same country.  $F_{Country/Total}$ , the fixation index due to the distribution of clusters into different countries, is tested by randomizing clusters (including all individuals) between countries.

**ACKNOWLEDGMENTS.** The authors acknowledge F. Kjellberg, F. Renaud, M. Choisy, and F. Prugnolle for helpful discussions and for their assistance in the analysis and interpretation of the results. We also thank 2 anonymous referees who considerably helped improve the manuscript. We are grateful to the Institut de Recherche pour le Développement and the Centre National de la Recherche Scientifique for financial support. The strains were isolated as part of a European Community STD3 project (n8T53\*-CT92–0129). This work was also supported in a framework of a French National Project ANR SEST.

- World Health Organization (2002) Leishmaniasis. Available at <http://www.who.int/zoonoses/diseases/leishmaniasis/en/>.
- Bañuls AL, Hide M, Prugnolle F (2007) *Leishmania* and the leishmaniasis: A parasite genetic update and advances in taxonomy, epidemiology and pathogenicity in humans. *Adv Parasitol* 64:1–109.
- Desjeux P (2004) Leishmaniasis: Current situation and new perspectives. *Comp Immunol Microbiol Infect Dis* 27:305–318.
- Lainson RS, Shaw JJ (1987) in *The Leishmaniasis in Biology and Medicine*, eds Peters W, Killick-Kendrick R (Academic, New York), pp 1–120.
- WHO (1998) *Leishmania*, geography. Available at <http://www.who.int/leishmaniasis/leishmaniasis.maps/en/index.html>.
- Tibayrenc M, Ayala FJ (2002) The clonal theory of parasitic protozoa: 12 years on. *Trends Parasitol* 18:405–410.
- Bastien P, Blaineau C, Pages M (1992) Leishmania: Sex, lies and karyotype. *Parasitol Today* 8:174–177.
- Bañuls AL, et al. (2000) Is *Leishmania (Viannia) peruviana* a distinct species? A MLEE/RAPD evolutionary genetics answer. *J Eukaryot Microbiol* 47:197–207.
- Bañuls AL, et al. (1997) Evidence for hybridization by multilocus enzyme electrophoresis and random amplified polymorphic DNA between *Leishmania braziliensis* and *Leishmania panamensis/guyanensis* in Ecuador. *J Eukaryot Microbiol* 44:408–411.
- Belli AA, Miles MA, Kelly JM (1994) A putative *Leishmania panamensis/Leishmania braziliensis* hybrid is a causative agent of human cutaneous leishmaniasis in Nicaragua. *Parasitology* 109(Pt 4):435–442.
- Da-Cruz AM, Machado ES, Menezes JA, Rotowitsch MS, Coutinho SG (1992) Cellular and humoral immune responses of a patient with American cutaneous leishmaniasis and AIDS. *Trans R Soc Trop Med Hyg* 86:511–512.
- Dujardin JC, et al. (1995) Karyotype plasticity in neotropical *Leishmania*: An index for measuring genomic distance among *L. (V.) peruviana* and *L. (V.) braziliensis* populations. *Parasitology* 110(Pt 1):21–30.
- Evans DA, et al. (1987) Hybrid formation within the genus *Leishmania*? *Parasitologia* 29:165–173.
- Hide M, Bañuls AL (2006) Species-specific PCR assay for *L. infantum/L. donovani* discrimination. *Acta Trop* 100:241–245.
- Kelly JM, Law JM, Chapman CJ, Van Eys GJ, Evans DA (1991) Evidence of genetic recombination in *Leishmania*. *Mol Biochem Parasitol* 46:253–263.
- Ravel C, et al. (2006) First report of genetic hybrids between two very divergent *Leishmania* species: *Leishmania infantum* and *Leishmania major*. *Int J Parasitol* 36:1383–1388.
- Balloux F, Lehmann L, de Meeüs T (2003) The population genetics of clonal and partially clonal diploids. *Genetics* 164:1635–1644.
- De Meeüs T, Lehmann L, Balloux F (2006) Molecular epidemiology of clonal diploids: A quick overview and a short DIY (do it yourself) notice. *Infect Genet Evol* 6:163–170.
- Chambers GK, MacAvoy ES (2000) Microsatellites: Consensus and controversy. *Comp Biochem Physiol B Biochem Mol Biol* 126:455–476.
- Lehmann T, et al. (1996) Genetic differentiation of *Anopheles gambiae* populations from East and West Africa: Comparison of microsatellite and allozyme loci. *Hereditas* 77(Pt 2):192–200.
- Russell R, et al. (1999) Intra and inter-specific microsatellite variation in the *Leishmania* subgenus *Viannia*. *Mol Biochem Parasitol* 103:71–77.
- Rougeron V, et al. (2008) A set of 12 microsatellite loci for genetic studies of *Leishmania braziliensis*. *Mol Ecol Notes* 8:351–353.
- Nei M, Chesser RK (1983) Estimation of fixation indices and gene diversities. *Ann Hum Genet* 47:253–259.
- Tibayrenc M, Kjellberg F, Ayala FJ (1990) A clonal theory of parasitic protozoa: The population structures of *Entamoeba*, *Giardia*, *Leishmania*, *Naegleria*, *Plasmodium*, *Trichomonas*, and *Trypanosoma* and their medical and taxonomical consequences. *Proc Natl Acad Sci USA* 87:2414–2418.
- De Meeüs T, Balloux F (2004) Clonal reproduction and linkage disequilibrium in diploids: A simulation study. *Infect Genet Evol* 4:345–351.
- Bartley D, Bagley M, Gall G, Bentley B (1992) Use of linkage disequilibrium data to estimate effective size of hatchery and natural fish populations. *Conserv Biol* 6:365–375.
- Vitalis R, Couvet D (2001) Estimation of effective population size and migration rate from one- and two-locus identity measures. *Genetics* 157:911–925.
- Waples RS, Do S (2008) LDNE: A program for estimating effective population size from data on linkage disequilibrium. *Mol Ecol Resour* 8:753–756.
- De Meeüs T, et al. (2007) Population genetics and molecular epidemiology or how to "debusquer la bete." *Infect Genet Evol* 7:308–332.
- Mark Welch DB, Meselson M (2000) Evidence for the evolution of bdelloid rotifers without sexual reproduction or genetic exchange. *Science* 288:1211–1215.
- Mark Welch DB, Meselson MS (2001) Rates of nucleotide substitution in sexual and asexually reproducing rotifers. *Proc Natl Acad Sci USA* 98:6720–6724.
- Pamilo P (1987) Heterozygosity in apomictic organisms. *Hereditas* 107:95–101.
- Suomalainen E, Saura A, Lokki J (1976) Evolution of parthenogenetic insects. *Evol Biol* 9:209–257.
- Gaffney D, Campbell RA (1994) A PCR based method to determine the Kalow allele of the cholinesterase gene: The E1k allele frequency and its significance in the normal population. *J Med Genet* 31:248–250.
- Nébavi F, et al. (2006) Clonal population structure and genetic diversity of *Candida albicans* in AIDS patients from Abidjan (Cote d'Ivoire). *Proc Natl Acad Sci USA* 103:3663–3668.
- Brookfield J (1996) Population genetics. *Curr Biol* 6:354–356.
- Paetkau D, Strobeck C (1995) The molecular basis and evolutionary history of a microsatellite null allele in bears. *Mol Ecol* 4:519–520.
- Pemberton JM, Slate J, Bancroft DR, Barrett JA (1995) Nonamplifying alleles at microsatellite loci: A caution for parentage and population studies. *Mol Ecol* 4:249–452.
- Mauricio IL, Gaunt MW, Stothard JR, Miles MA (2007) Glycoprotein 63 (gp63) genes show gene conversion and reveal the evolution of Old World *Leishmania*. *Int J Parasitol* 37:565–576.
- Regis-da-Silva CG, et al. (2006) Characterization of the *Trypanosoma cruzi* Rad51 gene and its role in recombination events associated with the parasite resistance to ionizing radiation. *Mol Biochem Parasitol* 149:191–200.
- Jackson JA, Fink GR (1981) Gene conversion between duplicated genetic elements in yeast. *Nature* 292:306–311.
- Morrisson AC, Ferro C, Morales A, Tesh RB, Wilson ML (1993) Dispersal of the sand fly *Lutzomyia longipalpis* (Diptera: Phlebotomidae) at an endemic focus of visceral leishmaniasis in Colombia. *J Med Entomol* 30:427–435.
- Balloux F (2001) EASYPOP (version 1.7): A computer program for population genetics simulations. *J Hered* 92:301–302.
- Tait A, MacLeod A, Tweedie A, Masiga D, Turner CMR (2007) Genetic exchange in *Trypanosoma brucei*: Evidence for mating prior to metacyclic stage development. *Mol Biochem Parasitol* 151:133–136.
- Martin-Sanchez J, Gallego M, Baron S, Castillejo S, Morillas-Marquez F (2006) Pool screen PCR for estimating the prevalence of *Leishmania infantum* infection in sandflies (Diptera: Nematocera, Phlebotomidae). *Trans R Soc Trop Med Hyg* 100:527–532.
- Rogers ME, Bates PA (2007) *Leishmania* manipulation of sand fly feeding behavior results in enhanced transmission. *PLoS Pathog* 3:e91.
- Akopyants NS, et al. (2009) Demonstration of genetic exchange during cyclical development of *Leishmania* in the sand fly vector. *Science* 324:265–268.
- Botilde Y, et al. (2006) Comparison of molecular markers for strain typing of *Leishmania infantum*. *Infect Genet Evol* 6:440–446.
- Rotureau B, et al. (2006) Diversity and ecology of sand flies (Diptera: Psychodidae: Phlebotominae) in coastal French Guiana. *Am J Trop Med Hyg* 75:62–69.
- Ben Abderrazak S, et al. (1993) Isoenzyme electrophoresis for parasite characterization. *Methods Mol Biol* 21:361–382.
- Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab Press, Cold Spring Harbor, NY).
- Goudet J (2002) FSTAT: A program to estimate and test gene diversities and fixation indices. Version 2.9.3.2. Available at <http://www.unil.ch/izea/software/fstat.html>.
- Wright S (1965) The interpretation of population structure by F-statistics with special regard to system of mating. *Evolution* 19:395–420.
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370.
- Goudet J, Raymond M, De Meeüs T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics* 144:1933–1940.
- Holm S (1979) A simple sequentially rejective multiple test procedure. *Scand J Stat* 6:65–70.
- Chevillon C, et al. (2007) Direct and indirect inferences on parasite mating and gene transmission patterns. Pangamy in the cattle tick *Rhipicephalus (Boophilus) microplus*. *Infect Genet Evol* 7:298–304.
- Goudet J (2005) HIERFSTAT, a package for R to compute and test hierarchical F-statistics. *Mol Ecol Notes* 5:184–186.