

Demographic history of Rats in Western Ghats

Humans have domesticated many different kind of animals for their own good foe eg,dog,cow,goat etc.But certain animals were not domesticated but still found their way living with humans, or became commensals. How did commensalism arose in animals that were not domesticated is a question worthwhile to ask. This being a bigger question, this study looked at a small subset of it, the demographic history of rats,which are best model organism to test this as they are small in size ,ubiquitous and are mammals.

Demographic history relates here to their effective population size, population expansion or population stabilization and colonization pattern if any. Population growth leaves characteristic signature in the genetic sequence of the species. This can be inferred from the pair wise genetic difference between the various genetic sequence of the individuals in the population(Rogers and Harpending 1992) and also by using Bayesian skyline plots(Drummond et al. 2005).

Recent studies have shown the rats of the south-Indian subcontinent not undergoing any kind of population expansion which have been stabilized long back in time(Aplin et al. 2011).An objective of the current study is also to test the same at a finer scale so as to get a detailed picture.

There are mostly two species of Rat found in Southern Western Ghats; *Rattus Rattus* and *Rattus satarae*.*Rattus rattus* cf. *rufescens* has a diploid chromosome number of 38 while that of *Rattus satarae* is 42(Pagès et al. 2011).In addition, they have distinct morphological differences. Commonly known, *Rattus rattus* is often brown or gray bellied found as commensal in human settlements and *Rattus satarae* having a pure white belly is the non-commensal, perhaps, forest dwelling species(Pagès et al. 2011).

It is known that *Rattus satarae* and *Rattus Rattus* are separate, sympatric species, with no gene flow between them and their similar external morphology is interpreted as the result of convergence through occupying the same ecological niche(Pagès et al. 2011). Pages et al acknowledged their limited sample size and better insights into the study could be provided by increasing the sample size. Through the initial pilot surveying, we did not find much result to support the same as all the rats caught in forest were, based on morphology, identified as *Rattus satarae* and most in human-settlements as *Rattus rattus*. This could mean that during our trapping season, *R. Rattus* were also present in the forest, but not captured by our trapping scheme, which was concentrated inside the confines of the forest. There might be some kind of niche partitioning between *R. Rattus* and *R. satarae* where *R. Rattus* is found only at the edge of the forest and *R. satarae* is found inside; leading to parapatry. Competition for resources is predictable between the two species as they are quite similar in size and inhabits. Further, it is also possible that the community in the forest itself has changed, since the data which was used in the study was collected in 1990; and that *Rattus rattus* has moved out of the forests and now lives entirely commensally with humans. Therefore, it is even more essential to understand the sympatric association of *Rattus* species in the forest, in view of further comparative studies on non – commensal and commensal populations.

Tissue samples of rats were collected from the Western Ghats from location including Kodaikanal, Kottagiri, Thaishola , ooty and Valparai. The samples were differentiated according to the places where they were found and also the habitat, e.g.; village, forest, plantation or forest edge.





For studying the demographic history of rats, entire Hyper variable region or d-loop (~900bp) as well as partial Cytochrome-b (~500bp) sequence were used as markers. Primers for d-loop were standardised,

EGL3H(rev)-5'CCATCTAAGCATTTCAGT,RJ2F(fwd)- 5'AGCCCATGATCAACATAACTG,EGL4L(fwd)-5'CCACCATCAACACCCAAAG,RJ3R(rev)- 5'CATGCCTTGACGGCTATGTTG.

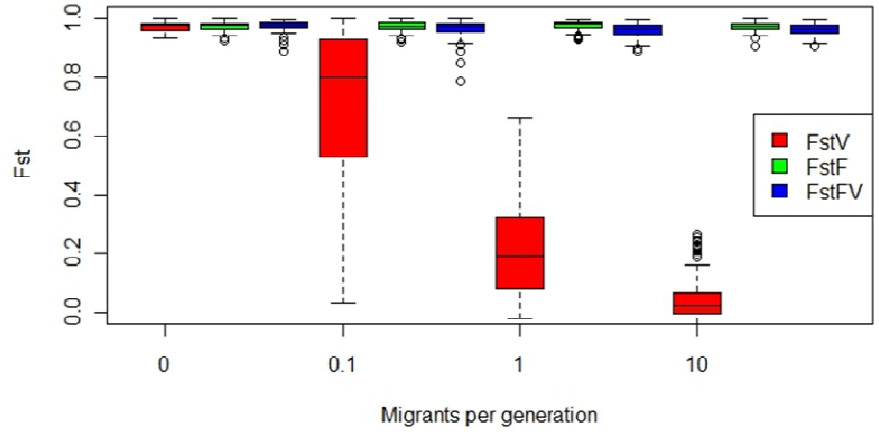
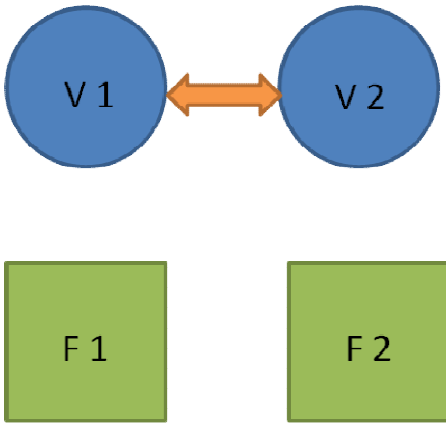
Primer for partial cyt-b are derived from Kocher's primers (Irwin et al. 1991) namely L14724-5'-CGAAGCTTGATATGAAAAACCATCGTTG and H15149-5'-AACTGCAGCCCCTCAGAATGATATTTGTCCTC.

DNA was extracted from samples using QIAGEN tissue extraction kit .

Phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version 5 (Tamura, Peterson, Stecher, Nei, and Kumar 2011). Based on the d-loop (509bp) and cytochrome-b (417bp) sequence of (~50) the individuals the Neighbour joining tree was built to verify the comparison between the coherence between the field ID using morphology and genetic ID using species-specific primers.

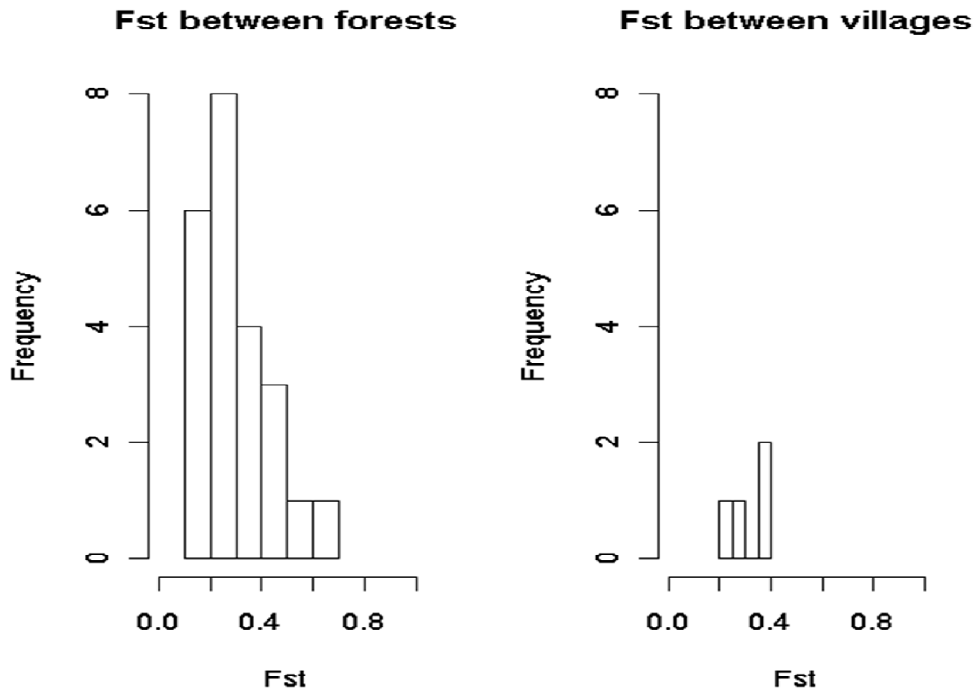
It is notable that there was some disparity between the phenotypic ID and the genotypic ID. This is particularly interesting and it would be worthwhile to see if there is some hybridization between two species by using microsatellite data over significant number of loci. Otherwise *Rattus rattus* of same morphology as that of *Rattus satarae* are present which might be due to extremely plastic phenotype as a result of sharing same ecological niche or a case of hybridisation which needs to be checked by karyotyping the individuals with inconsistency between field ID and genetic ID.

Fst is a measure of gene flow among populations. It is a measure of diversity among various alleles from different population. A simulation was done to understand how the Fst changes between the village and forest



population if there is gene flow between the villages but not among forests.

The simulation shows that the Fst between village decreases with increasing migrants per generation while the Fst between the forests remains constant because there is no migration between villages and forests. This was also checked by doing analysis on sequence data of d-loop of 509bp. The results of the data are shown below.



This shows that the frequency of Fst between the villages is quite low as compared to the frequency of Fst between forests. It shows that gene flow between forests is high as compared to gene flow between villages. This may be due to lack of connectivity between forests while villages are well-connected and rats are known to use passive modes of transport along with humans.

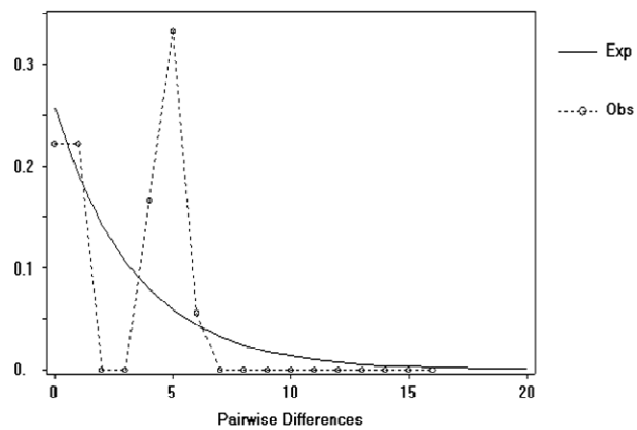
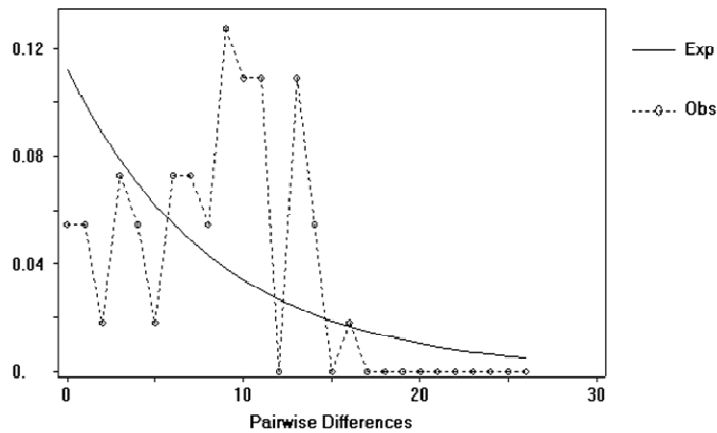
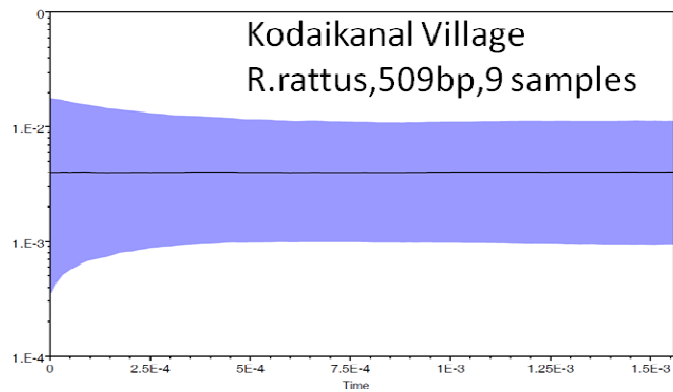
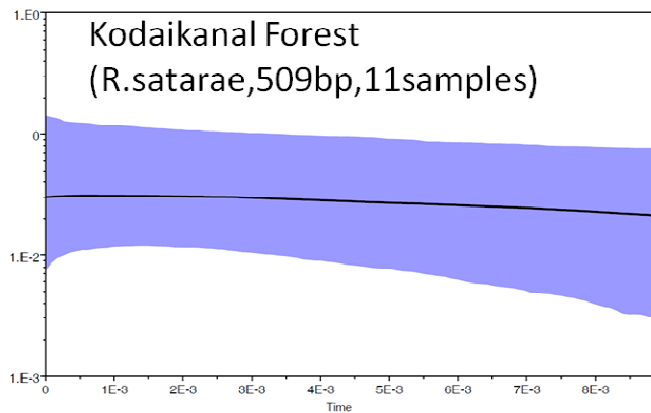
Rattus satarae

	KodaikanaEdge	Kodaikanlforest	KodaikanlPlantation	KottagiriEdge	KottagiriForest	kottagiriplantation	Ootyforest	ThaisholaEdge	Thaisholaforest
KodaikanaEdge	0								
Kodaikanlforest	0.06837	0							
KodaikanlPlantation	0.10109	-0.13098	0						
KottagiriEdge	0.41017	0.26937	0.48714	0					
KottagiriForest	0.21621	0.14314	0.17601	0.25105	0				
kottagiriplantation	0.29327	0.23375	0.66667	0.52656	-0.06378	0			
Ootyforest	0.34081	0.25592	0.66667	0.66416	0.45389	1	0		
ThaisholaEdge	0.10054	0.13268	0.11137	0.27541	0.19237	0.19034	0.30825	0	
Thaisholaforest	0.15668	0.12233	0.05686	0.32637	0.19422	0.25621	0.33675	0.03654	0

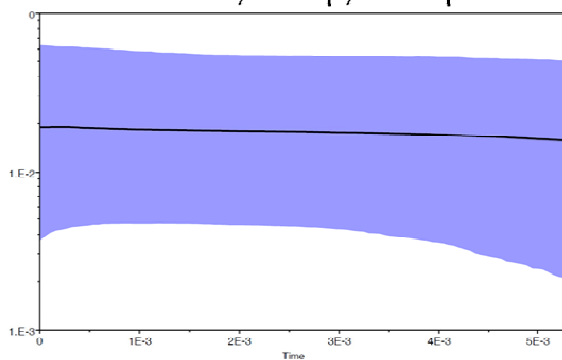
Rattus rattus

	KodaikanaVillage	KottagiriVillage	KottagiriPlantation	OotyVillage	ThaisholaVillage	ValparaiVillage
KodaikanaVillage	0					
KottagiriVillage	0.17479	0				
KottagiriPlantation	0.351	-0.03806	0			
OotyVillage	0.39744	0.07471	0.02628	0		
ThaisholaVillage	0.23404	-0.10174	0.06143	0.03448	0	
ValparaiVillage	0.26278	-0.03514	0.07646	0.00314	-0.03784	0

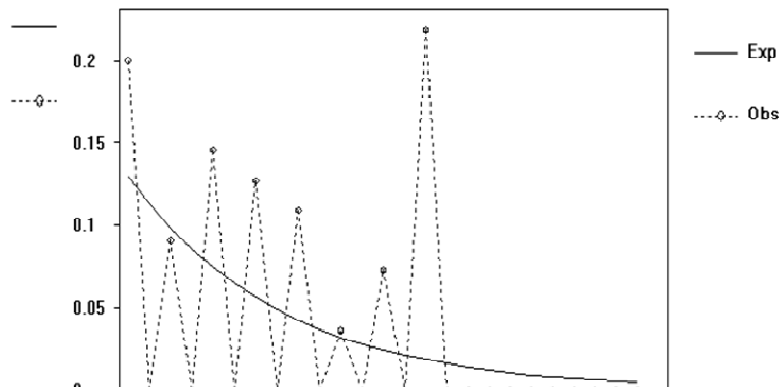
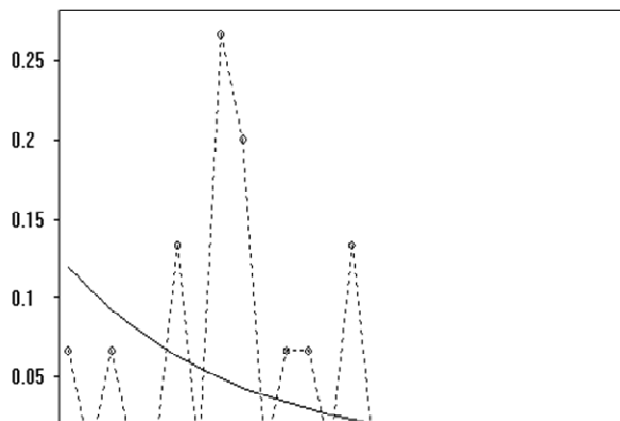
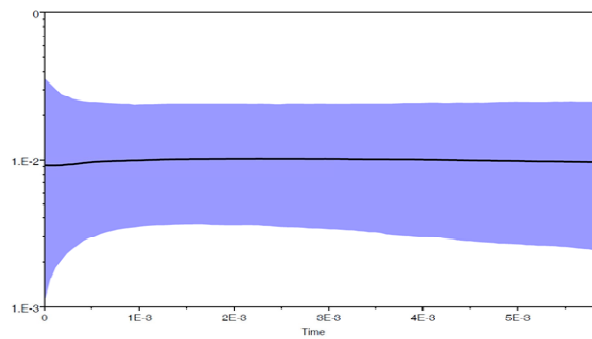
Demographic history of rats is important to study as their population sometimes back had expanded when rats started colonising human settlements. Rats now are found all over the world as commensals. We checked the demographic history of rat species using d-loop of 509bp as a genetic marker using BEAST program which uses Bayesian analysis to test if population size has changed in recent past. Demographic history was also checked using mismatch distribution(Rogers and Harpending 1992) using DNAsp 5.0(Librado and Rozas 2009).The result showed that population has not changed in recent past which is consistent with earlier studies(Aplin et al. 2011).



Thaishola village
R. rattus, 509bp, 6 samples



Thaishola forest
R. satarae, 509bp, 11 samples



Summary: This study shows that rat population has not expanded in recent past which may be due to their stabilization long back in time assaying for which will require different kinds of markers. It also shows the effect of well-connected villages and the transport system that has been utilised passively by rats to transfer their genetic material across populations, while the forests being ill-connected and continuously getting fragmented do not show any migrants across forest populations.