Why the Indian subcontinent holds the key to global tiger recovery

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Micro-evolutionary processes

Mutation → drift → migration

?
Current distribution

- Occupy ~7% of historical range
- ~3000-3500 tigers globally
- ~1500-2000 in India

Largest diversity of habitats: Indian subcontinent

What about genetic diversity?
Low diversity in India
Sampling from one location

Phylogeography

Luo et al., 2004
Assessing tiger genetic variation in the Indian subcontinent

Sampling: non-invasive scats from 73 tigers
28 protected areas including varied habitats

Mondol et al., 2009
Assessing genetic variation: mitochondrial DNA

\[ H_{SNP} = 1 - \sum_{i=1}^{n} p_i^2 \]

Ascertain for most variation
Total sequence length: 1263 bp for 4 regions

Mondol et al., PLoS gen 2009
Assessing genetic variation: nuclear DNA

STRs: High mutation rate, very polymorphic, independently evolving, co-dominant loci

Nuclear DNA from scat: degraded and low concentration

30 microsatellites from domestic cats, other tiger subspecies selected based on high heterozygosity and low allelic size range (<200 bp)

Fecal DNA microsats: possible genotyping and amplification error

All loci tested for amplification success with fecal DNA; 10 most consistent loci standardized; 5 of these loci used in Luo et al. for other subspecies

Each locus genotyped 4 independent times for each sample.

Final data includes samples with 75% or higher consistency
Genetic variation: mitochondrial DNA

32 haplotypes
76% genetic variation

Resampling simulations reveal that Indian diversity is not higher due to sample size

Mondol et al., PLoS gen 2009
Genetic variation: nuclear microsatellites

<table>
<thead>
<tr>
<th>Subspecies</th>
<th>Observed heterozygosity (S.D.)</th>
<th>Number of alleles (S.D.)</th>
<th>Allelic size range (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bengal (P. tigris tigris)</td>
<td>0.70 (0.16)</td>
<td>12.4 (3.6)</td>
<td>32 (7.7)</td>
</tr>
<tr>
<td>All other subspecies (Indo-Chinese, Malayan, Sumatran and Siberian)</td>
<td>0.53 (0.07)</td>
<td>7.2 (1.6)</td>
<td>16 (6.1)</td>
</tr>
<tr>
<td>All South-East Asian subspecies</td>
<td>0.56 (0.14)</td>
<td>7.2 (1.6)</td>
<td>16 (6.1)</td>
</tr>
<tr>
<td>(Indo-Chinese, Malayan and Sumatran)</td>
<td>Indo-Chinese (P. tigris corbetti)</td>
<td>0.57 (0.27)</td>
<td>6.2 (1.5)</td>
</tr>
<tr>
<td></td>
<td>Malayan (P. tigris jacksoni) and Sumatran (P. tigris sumatrae)</td>
<td>0.55 (0.05)</td>
<td>5.8 (1.5)</td>
</tr>
</tbody>
</table>

India holds 63% of global genetic variation

Mondol et al., PLoS gen 2009
Why are Indian tigers genetically more diverse?

2) High Population differentiation

3) High Ancestral effective size
Indian origin for tigers?

1) Paleontological data suggest South China origin

2) Phylogenetic data

3) Population genetic models: LAMARC

Mt DNA: MLE (m21) = 185 (44, 486); MLE (m12) = 0.19 (0.01, 59)
Nuclear DNA: MLE (m21) = 36 (31, 40); MLE (m12) = 13 (11, 15)

Tigers expanded their range into India

NO

Mondol et al., PLoS gen 2009
High population differentiation?

<table>
<thead>
<tr>
<th></th>
<th>North (n=10)</th>
<th>Central (n=11)</th>
<th>South (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>North (n=24)</td>
<td>0.027 (p=0.063)</td>
<td>0.041* (p=0.000)</td>
<td></td>
</tr>
<tr>
<td>Central (n=18)</td>
<td>0.236* (p=0.000)</td>
<td>0.019 (p=0.054)</td>
<td></td>
</tr>
<tr>
<td>South (n=26)</td>
<td>0.298* (p=0.000)</td>
<td>0.026 (p=0.279)</td>
<td></td>
</tr>
</tbody>
</table>

High differentiation for mtDNA

South and central India not differentiated

Structure contributes to high overall variation in Indian subcontinent

Mondol et al., PLoS gen 2009
Fischer-Wright coalescent: Constant population size

1. Reconstruct genealogical relationship between the samples
2. Distribute mutations on the genealogy:
   number of mutations proportional to branch length

Segregating sites: number of positions that differ across samples
Average pairwise difference: average number of differences between pairs of sequences

Low S, \( \pi \)
High S, \( \tilde{\pi} \)
Quantifying changes in population size with the coalescent
High Ancestral effective size?

Population decline quantified by other methods including LAMARC, m-ratio, BOTTLENECK
Decline ~ 200 years old

Mondol et al., PLoS gen 2009
How many tigers in Peninsular India?

• Effective population size: 23,280 (2,964, 151,008)
• Effective size / Census size = 0.4

\[ \downarrow \]

\approx 58,202 \text{ adult tigers (7,412, 377,520) in peninsular India 200 years ago} 

**Given current estimates: decline of 98%**

Mondol et al., PLoS gen 2009
Sensitivity analyses

• Does magnitude of decline change when more genetic loci are used?
  NO

• Is our result valid only for peninsular Indian tigers?
  NO
Alternate explanations for high diversity in India

• Greater extent of population decline for other subspecies?

NO

Mondol et al., PLoS gen 2009
Conclusions

• Indian tigers have high genetic variation

• This high variation is due to population differentiation and high ancestral size

• However, we have already lost around 98% of these tigers
Implications for conservation

The Indian subcontinent retains 50-60% of the global tiger population.....

living in varied and fragmented habitats.....

with 60-70% of species genetic variation.....

Proportion of global tiger habitat in India: 8-25%

Strong case for conservation of Indian tigers
Non-invasive genetic monitoring of tigers in Bandipur National Park

Molecular methods to identify tigers (Mukherjee et al., 2007)
Molecular sexing
Genetic individual identification and population estimation
Comparison to photographic mark-recapture estimates.

72 tiger scats
55 typed reliably at 5 loci
26 unique individuals (PID=0.005)
Genetic population estimate= 66 (13)
Photo-based estimate = 66 (13.8)
Future directions

- Sampling of historical skins to investigate ‘lost’ variation, better quantify decline
- Quantifying phenotypic variation: striping pattern
- Landscape level studies in high tiger density areas to investigate connectivity
What drives patterns of genetic variation in the Indian subcontinent: Geography, Climate, Ecology or Humans?

Uma Ramakrishnan
Global drivers of patterns of genetic diversity?

• Biogeographic divides

• Recent climatic fluctuations

• Ecology: dispersal ability and population size
Why the Indian subcontinent?

• Three major biogeographic realms (Palearctic, Africotropical, Indomalayan) intersect here
• Geologically interesting history
• Ecologically encompasses a diversity of habitat types
• Hominins have been present since the last million years
• Data poor
Where is the diversity in the Indian subcontinent from?

India part of secondary range expansion……..
In the Indian subcontinent

- No major biogeographic divides
  - Large differences in elevation across the subcontinent

- Impacts of climate not very clearly understood: Posters: Robin, Priya

- Ecology: different patterns for very large and very small species

- Significant anthropogenic impacts
How do we test these predictions?
Comparative framework

• Do differences in elevation matter?
  • Contrast species living in high elevations with closely related species in plains, Arunachal vs bonnet macaques

• Do differences in body size matter?
  • Contrast species across a range of body sizes, tigers, leopards, jungle cats

• Do differences in climatic regime matter?
  • Contrast ecologically similar species with small differences in habitat preference, leopard cat vs jungle cat

• Do anthropogenic impacts matter?
  • Contrast species that have been impacted negatively by humans (tigers) with unknown impacts (leopards)
Genetic patterns in two macaques

Largest primate genus

Most widespread primate
(after humans)

Most diverse distribution
(after humans)

Discovered in 2005
Identified as a genetically distinct species by us in 2007

Very high morphological and behavioural variation – species to individual
Highly adaptable to diverse environments
Mitochondrial DNA

Munzala phylogenetic tree and network

Strong signal of population differentiation in Munzala
Biological Sampling

n = 38

1540 km
>70% Bootstrap

network
Much less genetic differentiation than Munzala
A hint that the Palghat gap might be a biogeographic divide
Ecological parameters scale with body size

Carbone & Gittleman, 2002

Sutherland et al., 2002
Differences in body size

- **Jungle cat** (3 kg)
  - High density: Low movement
- **Leopard** (40 kg)
  - Intermediate density, movement
- **Tiger** (150 kg)
  - Low density: High movement

Graph showing relative density and relative dispersal with body size comparisons.
How do differences in density, movement impact genetic structure?

$N_e \sim$ Density/local population size
$m \sim$ dispersal

Low variation
high differentiation
Tiger?

High $N_e$
High m

Low variation
low differentiation
Leopard?

High $N_e$
Low m

High variation
moderate differentiation
Jungle cat?

Low $N_e$
Low m

High variation
low differentiation
Tiger?

High $N_e$
Low m

Low variation
high differentiation
Leopard?

High $N_e$
High m

Low variation
low differentiation
Tiger?
Differences in body size: isolation by distance across peninsular India

Sampling
Differences in body size: isolation by distance across peninsular India

Continuously distributed species reveal genetic patterns driven by body size.
Same body size, but different origins

Mukherjee et al., PLoS One, in revision
Length of tail as a percentage of head + body length in some cats

Means with ranges

20 30 40 50 60 70 80 90 100 110

Length of tail % of head + body length

Marbled Cat n=3
Clouded Leopard n=6
Asiatic Golden Cat n=3
Leopard Cat n=10
Rusty spotted Cat n=8
Black Footed Cat n=44
Jungle Cat n=10
Serval n=5
Caracal

Pocock (1939), http://www.abf90.dial.pipex.com/bco/ver4.htm

Mukherjee et al., PLoS One, in revision
Phylogenetic trees and haplotype networks

543 scats from various biogeographic zones
Used PCR-RFLP to ascertain jungle cats and leopard cats
Jungle cat: 55; Leopard cat: 40

Mukherjee et al., PLoS One, in revision
Population subdivision

Taxonomy (n)

<table>
<thead>
<tr>
<th></th>
<th>F. c. valbalala</th>
<th>F. c. kutas</th>
<th>F. c. affinis</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. c. valbalala (13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. c. kutas (23)</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. c. affinis (10)</td>
<td>0.20</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>F. c. prateri (9)</td>
<td>0.14</td>
<td>0.07</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Latitudinal range (n)

<table>
<thead>
<tr>
<th></th>
<th>10-19.9</th>
<th>20-28.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-19.9 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-28.9 (30)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>29-35 (10)</td>
<td>0.19</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Biogeographic zones

<table>
<thead>
<tr>
<th></th>
<th>Himalaya</th>
<th>N. East</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. East (9)</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>W Ghats (12)</td>
<td>0.90</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Jungle cat patterns are as expected
Continuous distribution throughout India
Weak isolation by distance

Leopard cat patterns in contrast to expectation
A clear break in geographical continuity indicated by genetic differentiation
Are we missing populations in-between in Central India?
If there is a gap in distribution, what is causing it?

Mukherjee et al., PLoS One, in revision
Are we missing populations in-between in Central India? If there is a gap in distribution, what is causing it?

Niche model analysis for leopard cat using climatic variables (temperature and rainfall)

*Mukherjee et al., PLoS One, in revision*
Leopard cat locations superimposed over the maximum temperatures in the warmest month.

Mean = 29.27°C
(95% CI: 28.59°C - 29.93°C; n = 217)

Mukherjee et al., PLoS One, in revision