GENETIC STRUCTURE OF A KEYSTONE HERBIVORE, *Tripneustes gratilla*, ON HAWAIIAN CORAL REEFS

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Common blooms of marine algae

Invasive alien algae can be found all around the main Hawaiian islands

Algal dominated

Photos: Jennifer Smith
"Phase shifts" on coral reefs

Top down effects: reductions in herbivore populations
- Overfishing
- Disease

Grazers’ functions:
- Conversion of primary production to fish-based trophic pathways
- Provision of suitable settlement substrata for new corals

Parrot fish
Surgeonfish
Urchins
Common on reef flats of the Indo-Pacific but inhabits variety of habitats

**Lessios et al. 2003**: little structure within major regions across the whole biogeographic range (CO-1 gene), but some populations showing significant $F_{st}$ values, especially the Hawaiian population.

A pattern of genetic differentiation without isolation by distance has been previously observed for other sea urchin species

*(Strongylocentrotus franciscanus: Edmands et al. 1996; Debenham et al. 2000)*

*(Strongylocentrotus purpuratus: Edmands et al. 1996; Flowers et al. 2002)*
The model species: Collector urchin *Tripneustes gratilla*

- *Tripneustes gratilla* are effective grazers on invasive red algae.
- Consume 7-10 grams algae per day (wet weight).
- Prefer invasive *Kappaphycus* and *Gracilaria* over native *Dictyosphaeria* and turf forming species (Conklin & Smith 2005).
Management

Keystone herbivores on reefs have high potential for larval dispersal

Urchin *echinopluteus* larvae
20-40 day development

Urchin *Tripneustes* larvae
20-30 days in a laboratory study in Japan
~ 42 days in the Philippines

What is the real larval dispersal of *Tripneustes*?
Connectivity among sub-populations determines management strategy.
The Questions…

- Sources and sinks: Are local populations subsidized by distant ones?
  - *Management application: Identify most productive areas for MPAs*

- Historical demography: Are populations expanding or contracting?
  - *Management application: Historical abundance can be used to set baseline*
Methods

Sampling (non-destructive)
Four main islands: Kaua‘i, O‘ahu, Mau‘i, Hawai‘i
Multiple sites within islands—windward vs. leeward
~ 40-50 samples / site
~ 750 urchin samples total

Estimate levels of connectivity with high-resolution markers: within and among islands
mt DNA: CO-I gene
microsatellite loci

Estimate long-term and recent demographic history
Sequenced 431 bp of mt CO-1 gene in 426 *Tripneustes gratilla*

- 42 polymorphic sites
- 47 haplotypes (33 unique)

$F_{st} = \frac{8}{105}$ significant tests out of 105 (15 sites)

Significance level = 0.05

**AMOVA:**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>% of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among islands</td>
<td>3</td>
<td>0.801</td>
<td>-0.00577 Va</td>
<td>-0.86</td>
</tr>
<tr>
<td>Among populations</td>
<td>11</td>
<td>9.567</td>
<td>0.00701 Vb</td>
<td>1.04</td>
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<tr>
<td>Within populations</td>
<td>411</td>
<td>275.625</td>
<td>0.67062 Vc</td>
<td>99.81</td>
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<tr>
<td>Total</td>
<td>425</td>
<td>285.993</td>
<td></td>
<td>0.067187</td>
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</tbody>
</table>
Tripneustes gratilla
Population structure

- Haplotype 1: n=83
- Haplotype 2: n=110
- Haplotype 3: n=87
- Haplotype 4: n=146
- Others < 2%

No significant $F_{st}$
Microsatellite development

Tripneustes gratilla

GIS (Genetic Identification Services, CA) identified 62 microsatellite-containing clones from four libraries, and developed primers for 39 microsatellite-containing clones.

Later, we isolated 24 more clones and developed primers for 14 of those microsatellites.

In total, out of those 53 primer sets, we selected 11 microsatellites for analyses, our choice based on reliability and polymorphism.

So far, 8 of them have been analyzed for part of the data set.
Tripneustes gratilla

Preliminary results: Genetic diversity

Linkage disequilibrium test
Our 8 analyzed loci are all evolving independently ($P < 0.05$)

Rich allelic diversity
12 to 36 alleles per locus (mean of 19.5)

Observed heterozygosity
ranging from 0.335 to 0.935, mean of 0.601 (± 0.050)

Hardy-Weinberg equilibrium
Under a $P < 0.05$ criteria, 61 tests out of 83 (8 loci x 11 sites) were significant for heterozygote deficiency
Only one loci does not show HWD
Corroborated by high $F_{IS}$ (mean = 0.293)

Common in marine invertebrate: Eastern Oysters (Reece et al. 2004)
Sea urchins (McCartney et al. 2004)
Tripneustes gratilla
Preliminary results: Population Structure

Absence of Population structure
No significant $F_{st}$, within or among island

No significant difference with NWHI

N=13   French Frigates
N=24   Johnston Atoll

Total of 319 samples

Sampling sites:
- N=24
- N=43
- N=20
- N=40
- N=39
- N=45
- N=42
- N=47
- N=37
Conclusions

High gene flow and connectivity among all the Main Islands

And maybe even with NWHI…?
Implications for management

Actual Hawai‘i Marine Life Conservation Districts

O‘ahu: Hanauma Bay
   Pupukea
   Waikiki

Hawai‘i: Keakakekua Bay
   Lapakahi
   Old Kona Airport
   Waialea Bay
   Waiopae Tidepools

Maui: Honolua-Mokule‘ia Bay

Lana‘i: Manele-Hulopo‘e

Molokini Shoal

LOCAL MPAs HAVE REGIONAL EFFECTS!!!
Implications for management

Use of *T. gratilla* to control the growth of macroscopic algae on shallow reefs (e.g. Kaneohe Bay)

According to those results, urchins from any location in the main Hawaiian islands could be transplanted to reduce the abundance of invasive algae.

There is the possibility to stock areas where *Tripneustes* suffered a decrease in population size.
Future work

- Finish the microsatellite genotyping for all populations and run the population structure analyses!

- Estimation of the effective population size and inference of the demographic history (bottleneck detection requires a minimum of 10 loci for reasonable statistical power)

- Apply the microsatellite approach to keystone parrotfish, *Scarus rubroviolaceus*
  
  16 polymorphic microsatellites have been developed so far
  
  Sampling will be done during the next year

- Apply this information to management strategy
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