

An external evaluation of the activities of INCT-CENBAM

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Executive Summary

- Conservation and utilization of the enormous biodiversity within the Brazilian Amazon demands a coordinated long-term approach to scientific monitoring and research. This is encapsulated by the objectives of INCT-CENBAN.
- The promotion of cross disciplinary collaboration is efficient and has fostered innovation.
- A reliable, easily accessible and widely utilised data depositary has been developed and, key to its success is investment in human resources.
- Developing relationships, skill sets and infrastructure in regional hubs is of fundamental importance and requires urgent financial support.
- Collecting long-term data sets needs done in a consistent manner and the RAPELD system under the auspices of PPBio is a sound approach.
- A few areas have been identified where efficiency can be enhanced, primarily through utilising molecular-based approaches and the excellent molecular facilities available at INPA.
- INCT-CENBAN provides a clear and innovative strategy to direct intellectual and capital investments in Amazonian biodiversity. Commensurate to its success is long-term stability through sufficient and on-going resourcing.

The overriding objective of INCT-CENBAN is one of conserving and utilizing Amazonian biodiversity and this is to be achieved by bringing together the plethora of scientific and technological disciplines that share this goal. This process alone improves efficiency because areas of duplication can be quickly identified, as are gaps in knowledge. It also has the by-product of stimulating innovation through fruitful inter-disciplinary collaboration – a form of ‘cross-pollination’ if you like. However, for effective conservation-based management, these diverse research activities need to be coordinated by a single entity that maintains a strict and long-term focus on applied outcomes. Furthermore, whilst maintaining a rigid set of objectives, this entity needs to interact at the community level, a component of which requires relationships to be fostered and skills developed, a long-term process. INCT-CENBAM is the entity placed to fulfil this role, and a logical requirement is institutional stability through appropriate resourcing. I summarise my observations of INCT-CENBAM below:

Human Resources

During my time with INCT-CENBAM it is clear that staff and students are highly motivated and, cognisant and open to the benefits of interdisciplinary integration. Indeed, it quickly became apparent that one of the strengths of INCT-CENBAM is its focus on human resources. For example, a critical component of interdisciplinary collaboration is easy access to data. Therefore a well developed data-base system into which researchers can deposit their meta-data is required. World-wide, the

establishment of data storage systems have been plagued by problems – including lack of consistency with data input, or the complete failure of researchers to contribute. It appears that these problems have been largely overcome by well placed and trained staff to facilitate the use of this system. Another impressive example is the investments that have been made to human resources within the regional hubs. Despite many activities being impeded by a lack of funds, alternative attempts have been made by locally placed staff to source the necessary support. This clearly demonstrates high levels of motivation, and alludes to healthy communication among the management structures of INCT-CENBAM.

Integrating Amazonian biodiversity research

Considerable effort has been directed towards developing the infrastructure and systems to allow for inter-disciplinary data synthesis and consistency of approaches over the long term. Key infrastructure developments that feed into this theme are the RAPELD/LTER plots of PPBio and the data management platform. Making use of the existing specimen storage facilities, and their further development is also planned. This is important as are the storage techniques. The stated desire of INCT-CENBAM that collections include the storage of tissues (of recorded provenance) in solutions amenable to molecular investigation (e.g. Ethanol or DMSO) is strongly supported. Molecular approaches are developing rapidly and are likely to become an increasingly important part of the ecological toolkit moving forward. There has also been work put into establishing connections with other data collecting organisations in order to broaden the scale over which a consistent approach to data collection and storage are applied. Further integration with other long-term monitoring systems (e.g. RAINFOR) would be beneficial. To complete the projects vision of ‘scientific and technological production chains’ further investment into infrastructure is required, especially within the regional hubs.

Technologies/processes that may improve efficiency with current projects

1/ Large investments in infrastructure and human resources for high throughput sequencing have been made overseas. Personal experience with one of these organisations (Korean-based MACROGEN) has been favourable, both in terms of cost and data quality. For routine sequencing I have found that access to MACROGEN has increased efficiency in my Australian-based genetics lab. However, high throughput sequencing facilities (and efficient genotyping facilities) are also present in the laboratory housing project ADAPTA. These technologies have the potential to be very beneficial to several projects of INCT-CENBAM, providing dedicated personnel are assigned to operate the machinery and disseminate the outputs (at a competitive cost).

2/ Routine sequencing for ‘Barcoding’ approaches (CO1 or 16sRNA) should be carried out to complement other taxonomic approaches.

3/ Rapid estimates of taxon richness (and perhaps diversity) for ‘micro-organisms using molecular approaches (especially terminal fragment length polymorphism and length polymorphisms at the internally transcribed spacer regions).

4/ Investigations of antimicrobial activity using a low cost miniature bio-assay that has been developed for this purpose. Papers detailing these approaches have been disseminated.

Potential Research Directions and Integration

During my time here at INPA I discussed research activities within numerous disparate fields. This provided opportunity to contribute to potential linkages between disciplines and to develop research ideas. I have met with the following staff and students (apologies to any I might have left off this list).

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|---------------------------|------------------------|
| 1/ Gabriella Zuquim | 17. Flavia Pezzini |
| 2/ Claudia Keller | 18. Cintia Rolin |
| 3/ Francisco Villamarin | 19. Michael Hopkins |
| 4/ Pedro Leitão | 20. Domingos Rodrigues |
| 5/ Flavia Costa | 21. Cecilia Nunes |
| 6/ Elizabeth Franklin | 22. Cristian Dambros. |
| 7/ Tania Sanoiotti | 23. Regina Luizao |
| 8/ Ted Feldpausch | |
| 9/ Carlos Alberto Quesada | |
| 10/ Marina Anciões | |
| 11/ William Magnusson | |
| 12/ Albertina Lima | |
| 13/ Jorge Porto | |
| 14/ Fabricio | |
| 15/ Roman Baptista | |
| 16/ Jose Julio Detoledo | |

I also provided two seminars:

1/ Title: Hypothesis driven drug discovery – antimicrobials and social insects (15th October)

Abstract:

The evolution of sociality in insects led to dramatic increases in group size so that colonies may consist of thousands or even millions of individuals, attracting the epithet ‘superorganism’. As this process produced colonies of animals living in very close proximity, we expect that there must have been an ever-present threat from contagious disease. Consequently we expect that mechanisms to defend against disease must also be a requirement of sociality. To test this hypothesis we examine cuticular antimicrobials on the surfaces of bees, wasps and thrips. For each of these groups, antimicrobial strength is examined from species ranging from solitary through to highly social.

2/ Title: Habitat connectivity and its influence on mating systems (14th October)

Abstract:

Habitat fragmentation is a major threatening process and knowledge of its implications to connectivity can contribute to conservation management. While genetic methods have contributed to our understanding of connectivity on evolutionary time-scales, measuring connectivity at a scale that is meaningful to the demography has been difficult to achieve. In this talk I discuss various approaches to measuring connectivity and the little studied implications of connectivity to mating systems.

Below I have provided summaries of some research themes and questions that have developed through discussions with staff at INCT-CENBAM (INPA) have not been prioritised in any way.

1/ Subterranean biodiversity

Subterranean biodiversity most likely has an important role in ecosystem functioning. Cataloguing components of subterranean diversity associated with PPbio plots is an important first step to assessing the ecosystem services provided by subterranean biota, and potentially, some impacts of environmental change. Soil fertility and the level of the water table have already been identified as important predictors of floristic characteristics and subterranean organisms are likely to play a role with this.

- Use of PPbio plots to measure alpha and beta diversity in components of subterranean microbial diversity (notwithstanding the technical challenges associated with these measures).
- a) Bacterial diversity can be characterized using Terminal Restriction Fragment Length Polymorphisms (TRFLP) of genes encoding 16S rDNA. Analyses of each sample are usually repeated in triplicate. Soil samples (500g) could be collected in sterile containers and refrigerated. Total DNA can be extracted using bead beating (commercial kits are available), 16S rDNA amplified using fluorochrome labeled primers f27 and r1492. Amplicons are then purified and digested with restriction enzymes (often *Hinf* 1 and *Rsa* 1). These digests can then be analysed on a modern capillary sequencer with taxon specific restriction sites resulting in specific combinations of terminal length (attached to the fluorochrome). Profiles or 'fingerprints' from all TRFLP analysis of samples can then be compared across plots and sites (using similar approaches to the INCT-CENBAM publication by Costa et al. 2005). The groups of bacterium involved could be identified by creating a 16S rDNA clone library from each sample. Approximately one hundred clones from each library would need to be sequenced in order to identify common taxonomic units. This approach would allow for a measure of phylogenetic diversity, which has certain advantages, however it is also very labour intensive and expensive. A similar approach to that described above could also be applied to highly abundant and diverse invertebrates for which taxonomic status is poorly understood (e.g. mites). Perhaps this approach could be applied in conjunction with 'barcoding' at Cytochrome Oxidase (sub unit 1).
- b) To assess fungal diversity The rDNA internal transcribed spacer (ITS) can be amplified using polymerase chain reaction (PCR) with primer pair SSU1758

(5'-GTCATTTAGAGGAAGTAAAAGTCG) and 58S8 (5'-CAGAACCAAGAGATCCGTTGTTG). Again these primers can be fluorescently labeled and because the resulting amplicons vary in length among taxonomic groups the number of size classes should reflect fungal diversity. The same analytical approaches as for the bacteria (point above) can then be applied.

- In order to make predictions on the impact of environmental changes perhaps diversity could be measured in areas with different soil types, plant communities and different rainfall patterns (the VR319 transect might be a good starting point?). There is also a topographic index of subterranean water that may also be useful.

2/ Genetic variation and adaptive potential

Within the Amazon basin a number of very well constructed genetic studies are investigating questions relating to genetic connectivity at broad and fine scales. However, largely because of the immense area of the Brazilian Amazon, information on levels of connectivity remains an impediment to the predicting the impacts of environmental change on biodiversity. Integration of data on connectivity with information collected as part of PPBio, and the broader activities of INCT-CENBAM, is needed to systematically associate environmental data with levels of connectivity and genetic variability.

Some specific questions that have emerged:

- a. Fluctuations in density of organisms in response to fire, is this a result of die off or migration?
- b. Can we assume that widely distributed species with high dispersal abilities lack genetic structure?
- c. How can the emerging technological advances with functional genomics integrate with ecological data to predict the consequences of climate change?

There has been recent interest in the application of functional genomics to understand processes at landscape levels. In contiguous habitat within the Amazon basin a major future impact is likely to be climate change with predictions of greater temporal variation and extremes of rainfall. For any one species resilience to these events is expected to be reflected by genetic variation and connectivity (dispersal ability). World wide, concomitant analysis of functional variation with typical assays of neutral variation (such as microsatellite variation) is lacking. The value of neutral genetic markers to assess dispersal is well recognised, and the statistical framework for such analysis well established. While neutral markers may also provide a reasonable estimate of functional variation, it is only a rough guide. Furthermore, analysis of functional variation can inform on different selective pressures in different areas, and potentially identify what those pressures are. These data, in combination with data on connectivity will be invaluable to predicting consequences of climate change within the Amazon basin. In place is a perfect system to investigate this.

Along the BR319 a transect of established ecological grids have captured data along an environmental cline. It is proposed that connectivity and 'landscape genomics' be assessed for a suite of species (belonging to a single taxonomic group) that are distributed along this transect. Species could be chosen to represent a range of natural history traits so that generalisations can be made and predictions made of the impacts on this group. Assessing adaptability and function genomics is a very well resourced research theme at INPA with the project ADAPTA. Excellent facilities are have been developed by project ADAPTA for extracting information on functional genomics and potentially to carry out manipulative experiments using the 'microcosm' set up.

3/ Cuticular antimicrobial compounds and colony size in ants. Ants are known to produce a series of antimicrobial compounds within several glands (esp. metapleural glands). Recent work is demonstrating that in some social invertebrates group size is a good predictor of antimicrobial strength. Assessing antimicrobial strength along this gradient of group sizes might establish this relationship for ants and feed into 'hypothesis-driven' bio-prospecting within the Amazon basin. Literature on appropriate assays has been disseminated to several staff within INCT-CENBAM.