Internet Appendix A36: Phytology

Figure A36.1 Illustrative Pitch Template Example

This pitch is reverse engineered from the paper:

Bohman, B., Phillips, R. D., Menz, M. H., Berntsson, B. W., Flematti, G. R., Barrow, R. A., Dixon, K. W. and Peakall, R. (2014). Discovery of pyrazines as pollinator sex pheromones and orchid semiochemicals: implications for the evolution of sexual deception. New Phytologist, 203(3), 939-952.

Pitcher's name	Marita Smith	For category	Phytology	Date completed	27/5/15
(A) Working Title	The chemistry of sexual deception in orchids				
(B) Basic Research Question	What are the organic compounds involved in the sexual deception strategy employed by orchids of the genus *Drakaea*?				
(C) Key paper(s)	Peakall, R. 1990. Responses of male Zaspilothynnus trilobatus Turner wasps to females and the sexually deceptive orchid it pollinates. Functional Ecology 4: 159-167 Bohman, B., Jeffares, L., Flematti, G., Byrne, L. T., Skelton, B. W., Philips, R. D., Dixon, K. W., Peakall R. and Barrow, R. A. 2012. Discovery of tetra-substituted pyrazines as semiochemicals in a sexually deceptive orchid. *Journal of Natural Products* 75: 1589 – 1594. Peakall, R. and Whitehead, M. R. 2014. Floral odour chemistry defines species boundaries and underpins strong reproductive isolation in sexually deceptive orchids. *Annals of Botany* 113: 341-355.				
(D) Motivation/Puzzle	Sexually deceptive orchids use volatile organic compounds to sexually lure pollinators. Specifically, these flowers lure the males of specific insect species by producing volatile compounds that mimic the female sex pheromone. As such, these orchids are characterized by highly specific pollination systems. Despite this specificity, this method of pollination has evolved independently on multiple continents, including Africa, Australia, Europe and South America. This is astonishing considering the wide range of chemical compounds involved in each specific flower-insect interaction. Of the few compounds that have previously been identified, it is apparent that a diverse range of chemical systems are involved in these interactions. How and why these unique systems evolved is not well understood. Very few of the volatile organic compounds involved have been identified and studied.				
THREE	Three core aspects of any empirical research project i.e. the "iDioTs" guide				
(E) Idea?	In order to more fully understand the phenomenon of sexual deception in orchids, it is necessary to examine the unique chemical systems involved and to identify the individual compounds responsible. The Australian orchid genus *Drakaea* and its specific relationship with the male thynnine wasp have never been examined in detail, providing a perfect study system. Using gas chromatography coupled to electroantennography (GC-EAD), it is possible to identify which of the many volatile organic compounds are detected by the insect antennae within a field environment. Once the compounds have been isolated, it is possible to chemically identify them using gas chromatography coupled to mass spectrometry (GC-MS). Then, bioassays in the field may be used to confirm the biological activity of individual compounds (or blends of compounds). Very little work has been done to determine the function of individual volatile organic compounds released by orchids. By combining the analytic capacity of several different disciplines, it should be possible to identify and test the activity of a range of volatile organic compounds.				

(F) Data?	- Sample collection of flowering specimens, 'baiting' for male thynnine wasps and collecting female thyninne wasps from adjacent shrubs - Analysis using GC-EAD to identify target compounds - Compound identification using GC–MS and NMR - Chemical synthesis of target compounds - Bioassays in the field using artificially presented flowers (as a measure of standard pollination) and the synthetic compounds dispensed onto a dummy (dressmaker's pin with a 4mm-diameter black plastic head) - Statistical analysis of results
(G) Tools?	- Analytical equipment (GC-EAD, GC-MS, NMR) and organic synthesis laboratory - Field equipment for bioassays during the short flowering period of the orchid (2 -3 weeks per year) - Statistical software
TWO	Two key questions
(H) What's New?	The compounds used by *Drakaea* for sexual deception have never been identified or produced synthetically in a laboratory environment. This study will provide novel data about the specific chemical mechanisms of the interaction between orchid and pollinator.
(I) So What?	Sexual deception in orchids has long been a scientific novelty. Very few orchid genera have been characterized, and it is likely that the chemical compounds involved are very diverse. The investigation of unusual pollination systems is important for the advancement of our understanding of the role of chemistry in evolution.
ONE	One bottom line
(J) Contribution	The primary source of the contribution will be a range of novel organic compounds responsible for the specific orchid-wasp pollination achieved using sexual deception by *Drakaea*.
(K) Other considerations	Is Collaboration needed/desirable? -Idea: no; -Data; yes –multi-disciplinary and multi-institutional preferred (will need to conduct bioassays in a range of environments) -Tools; yes –representatives and funding from various institutions Target journals – *Phytochemistry*, *New Phytologist* "Risk" assessment: -"no result" risk: low. GC-EAD should provide a full description of the range of volatile compounds pollinators are able to discern. By using a further battery of spectroscopic techniques, it is highly likely that these compounds will be successfully identified. -"competitor risk"(i.e. being beaten by a competitor): low. This study will require the cooperation of multiple disciplines across several institutions, requiring the input of several experts. -risk of "obsolescence": Low. A study of this depth and magnitude has not been attempted in Australia before.