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The Culture of a "Four Species" *Haliotis* Hybrid in a Marine Shellfish Hatchery

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ABSTRACT The hatchery culture of a previously unknown hybrid abalone combining the parentage of four species of *Haliotis* is described. The parent specimens, both hybrids themselves, were a female *H. rufescens* Swainson, 1822 × *H. sorenseni* Bartsch, 1940, and a male *H. corrugata* Wood, 1828 x *H. walallensis* Stearns, 1899. Details of the technique used to accomplish this, and the problems encountered during the relatively difficult culture of this multi-species hybrid, are discussed. Shell specimens of this exceptional cross are described and illustrated with high-resolution color photography, and the pigmentation and morphology of the soft body parts are described. Some possible reasons why such an unusual hybrid would not likely be found in natural populations are advanced.

INTRODUCTION

Hybridization in natural populations of Eastern Pacific *Haliotis* is well documented (Owen et al., 1971; Owen and Potter, 2002, 2003; Owen, 2004), as is the culture of some of these hybrids in marine biological laboratories (Owen and Meyer, 1972; Leighton and Lewis, 1982; Leighton, 2000). Instances of a number of these hybrids proving to be fertile in laboratory culture have also been described in the literature (Owen and Meyer, 1972; Leighton and Lewis, 1982). The study described here was part of a series of investigations by Owen which demonstrated for the first time that hybrids among abalone species do, in some combinations, produce viable progeny which become reproductively competent upon reaching sexual maturity. At least two, and probably three, different multi-species hybrids, resulting from the crossing of a fertile two-species hybrid with a third species, have also been documented from natural populations (Owen, MS in preparation). In the latter instance, only single specimens are known to exist of each type; their identity confirmed by the morphology of both shell and soft body parts. Such hybrids are extremely rare, and only due to a huge amount of shell material retrieved and carefully surveyed in abalone processing plant discard piles has evidence of interbreeding been detected. The commercial harvest of abalones has afforded an opportunity to examine very large numbers of individuals, and a comparable sample size is available for few other shellfish families.

Throughout 14 years, from 1965 to 1979, the author was employed by a marine shellfish hatchery, (Pacific Mariculture, Pigeon Pt., California). This position provided the opportunity to work with living animals of the different West Coast *Haliotis* species, including a number of rare hybrids. These were obtained from the author's own diving efforts, and from donations of commercial diver friends with whom he had worked (Owen, 2004).

Initial work with conspecific fertilizations led to more advanced and complicated multi-species hybrid crosses and culture, which was highlighted in 1971 with the production of the four species hybrids described herein.

MATERIAL AND METHODS

Animals used in all spawning and culture experiments were kept in fiberglass-covered plywood tanks of 400 liter capacity measuring 0.7 x 1.0 x 1.2 m. Seawater, pumped directly from a near-shore intake adjacent to the hatchery, ran through the tanks continuously, and flowed back into the sea through a discharge pipe located ~200 m distant from the intake (known as an "open circuit" or through-flow system). At this point in time, methods used today to induce spawning in abalones were unknown. Early abalone hatchery operators relied on spontaneous spawnings (Leighton, 2000). When specific sea conditions associated with "spawning cues" (see Discussion, Remarks) were observed just prior to this spawning, the hybrid parents were given a quick prophylactic "fresh water rinse" (not sea water) and placed in 20-liter plastic pails which were first sterilized with a mild chlorine solution and rinsed copiously with fresh water (not seawater). Highly filtered (<1.0 micrometer) ultraviolet-irradiated seawater was then very slowly run into the two well-separated pails containing the hybrid parents to be spawned. (Prior to the spawning, both hybrids were examined and found to have very mature gonads and deemed ready to spawn).

RESULTS

Approximately one hour after the male *H. corrugata* × *H. walallensis* was exposed to the water-mass that had been filtered, UV-irradiated, and now contained the "natural spawning cue", it started to spawn. Thirty minutes later, the female *H. rufescens* x *H. sorenseni* started spawning as well. After 30 minutes of copious spawning, the eggs were very gently poured off the female and gently rinsed on a 48 micron "Nitex" (nylon) screen with the same <1 micrometer-filtered UV-irradiated seawater. The eggs were then separated into two groups of approximately 20,000 each, and suspended in two 10-liter plastic containers filled with this same highly filtered and UV-treated seawater. One pail was then set aside, well away from the second container, in another part of the hatchery. This was saved as an "unfertilized control" to inspect later for evidence of spurious fertilization (e.g., cell division) which would indicate unwanted sperm had contaminated the culture. The contents of the second pail were then fertilized with 25 ml of a diluted suspension of "sperm water" from the pail containing the male *H. corrugata* × *H. walallensis* hybrid.

An inspection of approximately 300 eggs from the unfertilized control two hours after the spawning showed no fertilization. Similar results were noted after 6 and 24 hours. It was clear that no spurious fertilization had occurred, and after about 30 hours the control culture was discarded.

An inspection of approximately 300 of the fertilized eggs after 30 min showed many motile sperm cells, but they appeared to become very sluggish and feeble upon traversing the jelly coat and approaching the egg membrane. Further, few eggs showed signs of being fertilized, and normal cell division (mitosis) seemed well under 1%. Inspection of conspecific crosses being conducted elsewhere in the hatchery (*H. rufescens*, and *H. kamtschatkana assimilis* Dall, 1878) exhibited normal rates of fertilization (~100%) and normal early cell division. Twenty-four hours after fertilization, it appeared about 1% of the eggs had become rotating trochophores, but many appeared oddly shaped and abnormal. After 48 hours, an inspection of a few (15-20) swimming larvae showed mostly normal appearing veliger larvae which had undergone torsion, but there appeared to be only about an estimated 250 swimming in the entire culture. This number seemed to drop a bit by the fourth day (estimate 150-200), and as they were approaching late veliger stages and becoming benthic in what appeared to be a fairly normal manner, they were introduced to a water table coated with diatoms with the hope that possibly 75-100 might survive to become juveniles. This occurred, and postlarval development proceeded extremely well, suggesting the possibility that "hybrid vigor" was being expressed. Excellent survivorship continued, and at about two months, the group progressed through the sometimes problematic "1st respiratory pore stage" of development with very little mortality It is just prior to this point that many conspecific postlarvae suffer a significant mortality rate, or "postlarval attrition" (Leighton, 2000).

Over the two years following the date of the two-hybrid spawning, the animals grew well, albeit at very different rates, which is common with conspecific normal crosses as well. As the larger animals of the group passed 25-30 mm in shell length (12-13 months), morphological characteristics of all four species could be observed in shell and animal bodies throughout the group. Numbering about 50 specimens as the group approached two years of age, the largest ~25% had grown to 70-75 mm, and the morphology of all four parent species became even more evident throughout the group, often combined into individual specimens in a highly variable manner. Unfortunately, at approximately 38 months of age, an aquarium accident terminated the experiment.

Due to the dramatic visual demonstration of all four species in the pigmentation and markings of the animals, all individuals of the entire group had been photographed just prior to the accident (as a photo assignment by a hatchery employee studying photography). Unfortunately, the photos were not available at the time of this writing. A group of 23 of the shell specimens are illustrated on Plates 1 and 2.

DISCUSSION

One of the more striking findings of these hatchery experiments is the high fertility rate of not only all the species of West Coast *Haliotis*, but of many, if not most, of their hybrids as well (Owen et al. 1971; Owen and Meyer, 1972; Leighton and Lewis, 1982; Owen and Leighton, 2002). Fertilization rate is largely dependent on gamete condition and concentration (Leighton and Lewis, 1982; Leighton, 2000). This appears to be true of many of the species of South Australian *Haliotis* as well, though it is not currently known if the hybrids of these species are fertile (Owen and Kershaw, 2002; 2003). It is curious that although fertilization and subsequent development of the fertilized gametes and early larval stages was largely abnormal in the instance reported here, another multi-species hybrid of nearly the same parentage provided an example of extreme contrast (Owen and Meyer, 1972). Early in 1969, Owen cultured a hybrid of a male *H. rufescens* × *H. sorenseni* hybrid crossed with a female specimen of *H. corrugata*. The techniques used in this spawning were similar to those employed in the four-species cross; however, in this instance, the percentage of eggs fertilized appeared to be virtually 100%, and subsequent larval development proceeded

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normally. Most significantly, the larvae had a survivorship through the difficult physiological process of becoming benthic and subsequent metamorphosis of over 95%. As stated above, it is at this point a substantial die-off often occurs with conspecific crosses. Postlarval attrition is frequently in excess of 90%. The difference in these two multi-species crosses may be due to the fact that the sex of the *H. rufescens* × *H. sorenseni* hybrid was inverse: in the highly successful cross the hybrid parent was a male. In some instances, heterospecific crosses that yield low fertilization rates succeed more when the reciprocal cross is made (Leighton and Lewis, 1982). It may well be that had the sexes been reciprocated in the four species cross, these early stages of development might have proceeded quite differently. However, obtaining living specimens of *H. corrugata* × *H. walallensis*, of either sex, unless produced artificially, would prove to be most difficult as naturally occurring examples of this hybrid are extremely rare (Owen et al. 1971; Owen and Potter, 2003).

The animals of the four-species hybrid group were highly variable in body pigmentation and epipodial features. This is no doubt due to the fact that the four parent species vary broadly in these morphological aspects as well. Of interest is the fact that no particular pattern of animal characteristics seemed to be found in any particular shell. For example, an animal having body and tentacle pigmentation appearing most like *H. corrugata*, with epipodial papillae most resembling *H. walallensis*, might be found in one of the five or six larger shell specimens most resembling *H. rufescens*. However, other shell specimens most resembling *H. rufescens*, but with slightly stronger spiral ribbing, or more of a suggestion of weak folded ridges, might possess soft body parts that appeared entirely different. This condition appeared throughout the entire group, with patterns of body and tentacle pigmentation, details of epipodial margin and papillae structure, appearing in random order throughout all animals, regardless of the morphology of the shell containing the animal‘s body. As stated in “Results”, this phenomenon was so bizarre and pronounced that all individuals of the entire group were photographed by a hatchery employee studying photography as a hobby (John Lanzone, current address unknown) shortly before the aquarium accident ended the experiment.

It is exceedingly unlikely that a four-species hybrid would ever be found in natural populations for a number of reasons; the foremost being that if a female two-species hybrid spawned, the odds against it being both physically isolated from all other *Haliotis*, but in the presence of a spawning male hybrid of dissimilar parentage, would be impossibly high.

A number of the shell specimens have the weak, fading, “orange band” found in some juvenile specimens of *H. sorenseni* and *H. rufescens* × *H. sorenseni* hybrids (Owen, 2004) indicating the female *H. rufescens* x *H. sorenseni* parent may have such a band, but erosion in the early stages of shell growth prevents this from being detected. The presence of a strong “orange band” in a shell specimen most resembling *H. rufescens* is extremely curious, and may possibly represent a recessive gene for this character.

REMARKS

Natural cues that promote coordinated spawning in abalone and other benthic, broadcast–spawning, marine invertebrates are not well understood. It is considered instructive to relate events associated with uninduced spawnings in groups of abalones held in tanks at Pigeon Point. Spontaneous mass spawnings appeared to be correlated with a slight increase in temperature and pH of the seawater, conditions that were usually associated with periodic southerly winds and were observed most often between June and October. These events almost always occurred during the full or new moon (generally a day before to a day after). Typically, the prevailing northwest wind would cease, and a gentle southerly breeze (8-15 kph) would develop. A few hours after the wind reversal, a very clear, blue, water mass would become visible, moving from offshore towards the mainland, with an abrupt line separating it from the usual relatively turbid (upwelled) water generated by the northwest wind. This clear water would displace the turbid water, and shortly after would be pumped up into the hatchery tanks. The temperature would be elevated a few degrees (typically from approximately 10.0-10.5°C, to 14.0-14.5°C), and the pH would rise about 0.5 unit to approximately 8.3. From cursory observations, it seemed that the offshore water had a very different planktonic composition, and the plankton would rather quickly block the filtration devices, with the accumulated material on the filters possessing an acrid odor reminiscent of fresh-cut cucumbers. This incoming seawater was rich in dissolved organic material (probably algal metabolites) and when heated to 25-30 C with a heat exchanger, a thick foam would be produced in the tanks, accompanied by the intense "cut cucumber" odor mentioned earlier. Such phenomena were never observed during periods of typical northwest weather.

Lunar periodicity in spawning of abalone and a host of other marine organisms is well recognized. The sea conditions related here also may have introduced chemical factors that acted in concert to synchronize gamete release throughout mixed species groups held in the hatchery seawater tanks. In the four-species hybrid study reported here, ultraviolet light was used to sterilize the incoming seawater, and possibly provided an additional spawning stimulus, as UV-irradiation is now known to facilitate spawning in abalone (Leighton, 2000). However, it is strongly believed that the main impetus causing spawning during these lunar periodicity events was the radically different water mass described above, as the animals in the main tanks receiving raw non-UV treated water spawned copiously as well.

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REMINDER

The Southern California United Malacologists (SCUM) Annual Meeting will be held January 24, 2015 in the Heritage Room of the Laguna Hills Community Center and Sports Complex 25555 Alicia Parkway, Laguna Hills. For more information contact Carol Stadium at: fossilreef1@ATT.net.

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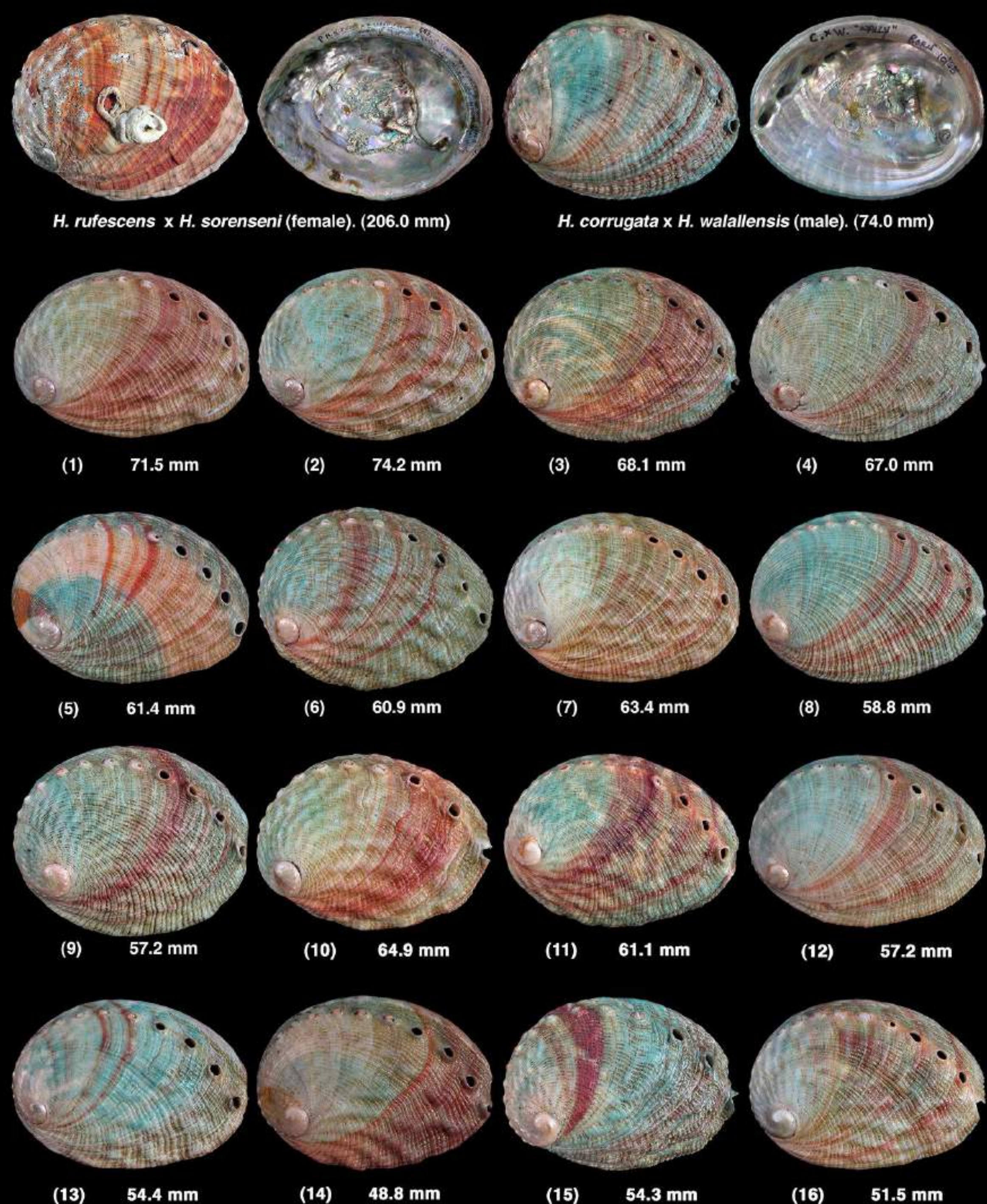

Plate 1.

Top Row: Actual "Parent" specimens of "4 Species" hybrids.
Rows 2-5: "4 Species" hybrids.

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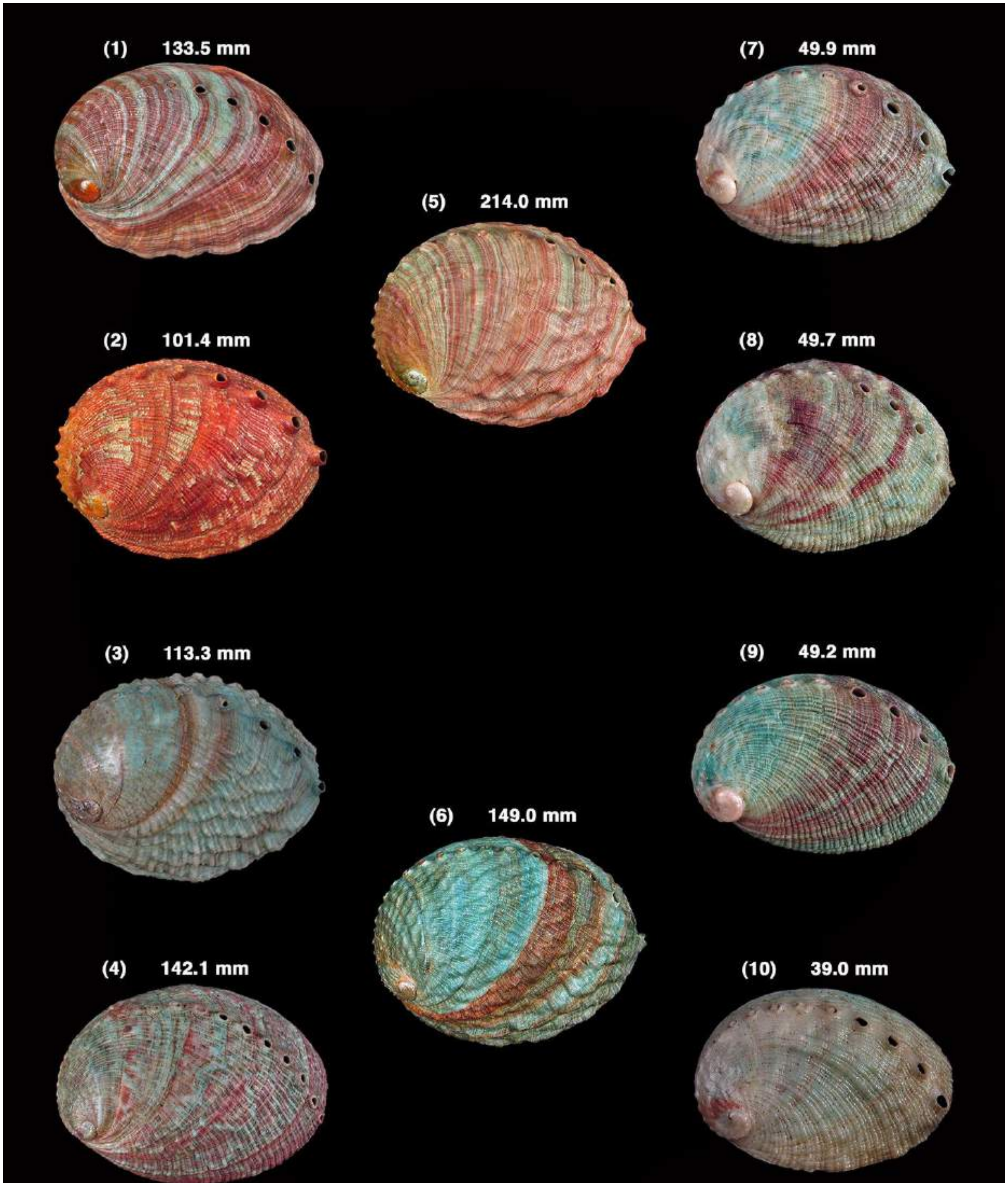

Plate 2

Left row: (1) *H. rufescens*; (2) *H. sorenseni*; (3) *H. corrugata*; (4) *H. walallensis*
Center row: (5) *H. rufescens* x *H. sorenseni*; (6) *H. corrugata* x *H. walallensis*
Right row: (7) through (10): "4 Species" Hybrids