ABSTRACTS OF PAPERS
Fifteenth Annual Albert L. Tester Memorial Symposium
5–6 April 1990

The Albert L. Tester Memorial Symposium is held in honor of Professor Albert Tester, who, at the time of his death in 1974, was senior professor of zoology at the University of Hawaii. The faculty and students of the Department of Zoology proposed an annual symposium of student research papers as a means of honoring, in a continuing and active way, Dr. Tester's lively encouragement of student research in a broad range of fields within marine biology. Papers reporting original research on any aspect of biology are solicited from students at the University and these papers are presented at the Symposium, which takes place during the spring semester. Income from contributions to the Albert L. Tester Memorial Fund at the University of Hawaii Foundation is used to provide prizes for the two best papers judged on quality, originality, and importance of research reported, as well as the quality of the public presentation. The Waikiki Aquarium presents the Michael Weekley Award, based on the same criteria. Judges include members of the faculty of the Department of Zoology, other departments within the biological sciences, and winners of the awards from the previous year's symposium. In addition, a distinguished scholar from another university is invited to participate in the symposium as a judge and to present the major symposium address. This year Corey S. Goodman of the Department of Molecular and Cell Biology and Investigator, Howard Hughes Medical Institute, University of California, Berkeley participated in the symposium.

Costs and Benefits of Parasitism in a Coral Reef System

Greta S. Aeby

The life cycle of the digenetic trematode Plagioporus sp. includes an intermediate stage that encysts in the coral Porites compressa and an adult stage that probably resides in a coral-feeding fish. Strategies used by both parasite and its hosts to maximize individual fitness are often conflicting and must be balanced for a stable parasite/host system to develop. I investigated potential costs and benefits to the Plagioporus sp. parasite and its hosts that could lead to a stable parasite/host system. Infected coral polyps appear as swollen cysts and have lost their ability to retract into their calices. The polyps' altered appearance and behavior might allow fish to minimize parasitism by avoiding the cysts or to maximize feeding efficiency by preferentially feeding on the cysts. The fishes' response to the infected coral was tested in the laboratory by offering a corallivorous butterflyfish, Chaetodon multicoloratus, a choice of either parasitized or unparasitized P. compressa. Chaetodon multicoloratus exhibited a preference for parasitized coral, which received 83.3% of the total bites ($P < .0001$). It was predicted that parasitism would reduce the growth rate of P. compressa. Unparasitized coral showed a significant difference in growth from parasitized coral. Parasitized coral had an almost 50% reduc-
tion in growth. Predator exclusion cages were used to determine if predation was a factor in the reduced growth rate and also if it affected the parasite levels of infected corals. No significant differences were found in growth between caged and uncaged corals. The uncaged parasitized corals showed a marked reduction in level of parasitism ($P = .0075$), and the infected polyps were replaced with healthy ones. This suggests that parasite removal is beneficial to the coral and also to the parasite by increasing its rate of transmission.

Sequence Analysis of a Phosphoenolpyruvate Carboxylase (PEPC) Gene from Sugarcane and Comparison with a C-4 Specific PEPC Gene from Maize

HENRIK H. ALBERT

PEPC catalyzes the first step of carbon fixation in C-4 plants, which include several species important in tropical agriculture. To study this gene, a genomic library has been constructed from *Saccharum* hybrid variety H32-8560, and five independent clones positive for PEPC have been isolated and purified. In maize, a C-4 specific PEPC has been shown to be 5.3 kb long, with 10 exons and 9 introns. We have determined much of the sequence of a sugarcane PEPC gene from just upstream of the putative translation start codon to exon number 9. Exons that have been sequenced entirely are identical to the maize gene in length and position, but both nucleotide and amino acid sequences are only ca. 2/3 identical to the maize sequence. Introns are in the same position and splice sequences are conserved, but in general nucleotide sequence and length of introns show no similarity to the corresponding maize introns. Exon 1 is quite similar to the maize sequence at its 3' end, but significantly different at the 5' end, with the initiation codon 12 nucleotides downstream relative to the maize site. Sequences within 200 bp upstream of the start codon show no similarity to the maize untranslated leader or upstream regulatory sequences.

Sodium Channel Activation Mechanisms: Insights from $\Delta^9$-Tetrahydrocannabinol

DANIEL A. ALICATA

Strichartz et al. (1978, J. Pharmacol Exp. Ther. 207:801–809) have reported that $\Delta^9$-tetrahydrocannabinol (THC) slows sodium channel activation and suppresses ionic conductance ($g_{Na}$) in a voltage-dependent manner in frog nodes of ranvier. In addition those authors reported that channel inactivation processes were not affected by THC action. I have repeated this work in crayfish giant axons and confirm that channel activation is slowed by THC. However, I find that inactivation is not spared from THC action. Furthermore, conductance-voltage curves were unaffected by THC treatment, indicating no voltage-dependent changes in ionic conductance. Additionally I have investigated the effects of THC on gating current ($I_{gON}$), tail currents, and secondary activation. These results provide new evidence associating gating current mechanisms with sodium channel behavior.
Food Resources for Larval Growth: Patches and Picoplankton

JANICE L. BELL

For marine invertebrates, larval growth rates in the plankton impact dispersal, recruitment, and the timing of metamorphosis. Previous laboratory studies of gastropod larvae have suggested that adequate growth is achieved only when concentrations of algal food are much higher than phytoplankton concentrations in the ocean, especially those in oligotrophic environments. Effects of larval concentration, food diversity, phytoplankton concentration, and picoplankton abundance on larval growth and survival were evaluated for the prosobranch gastropod Crepidula aculeata. Larvae were grown on particles in natural seawater or on single- or multiple-species diets of the laboratory-cultured algae Tahitian Isochrysis galbana, Nannochloris sp., and Chaetoceras gracilis. Larval growth rate increased with the number of algal cells available per larva. Highest growth rates were achieved at the lowest larval concentration (20 larvae/liter), and larvae in this low concentration grew, even with the low algal concentrations present in natural seawater. When algal concentration and diversity of algal cell types were compared, larval growth rates were not influenced by diversity; total algal biomass as indicated by cell volume had a stronger effect. Larvae grown on particles from natural seawater demonstrated higher growth rates and percentage survival than larvae grown on cultured-algal diets of the same particle concentration as the seawater. Two factors in natural seawater, picoplankton and availability of patches, may contribute to its superiorit as a food resource. Various particles in seawater were separated by reverse filtration, and ambient phytoplankton particles (> 2 μm) were concentrated (three times) to simulate patches. Larval growth rates in the concentrated fraction were higher than those in ambient seawater. In oligotrophic oceans, picoplankton, low larval concentration, and phytoplankton patches may combine to enhance larval growth and survival.

Rapid Inhibition of Prolactin Release by Cortisol from the Pituitary of the Tilapia, Oreochromis mossambicus, Accompanied by Reductions in cAMP and 45Ca++ Accumulation

RUSSELL J. BORSKI

Prolactin (PRL) and cortisol (F) are thought to play central roles in the freshwater and seawater (SW) osmoregulation, respectively, of many euryhaline fishes, including the tilapia, Oreochromis mossambicus. In vitro studies have shown that small physiological changes in osmotic pressure (OP) rapidly alter PRL release from the tilapia rostral pars distalis (RPD), with release increasing as OP is reduced. Our present findings demonstrate that physiological concentrations of F exert a dose-related inhibition of PRL release stimulated by exposure to reduced OP during 18- to 20-hr incubations. Inability of other steroids (testosterone, estradiol, and cholesterol) except for dexamethasone, a synthetic glucocorticoid agonist, to mimic effects of F on PRL release suggests that F's action is specific to this SW-adapting hormone. Perfusion
studies indicate that F inhibits PRL release within 20 min \((P < .001)\). The rapid nature of F’s action seems to preclude genomic mediation normally associated with steroid hormone action. Therefore, the question of whether F’s action was mediated by the second messengers, \(\text{Ca}^{++}\) and cAMP, was tested. Addition of 50 nM F significantly reduced accumulation of cAMP and \(^{45}\text{Ca}^{++}\) in the tilapia RPD within 15 min. Furthermore, the \(\text{Ca}^{++}\)ionophore A23187, which raises intracellular [\(\text{Ca}^{++}\)], overcame the inhibitory effect of F on PRL release. Taken together, these findings suggest that F may have a physiologically relevant role in the rapid modulation of PRL secretion in vivo and that its inhibitory action on PRL release may be mediated, in part, through its ability to inhibit intracellular cAMP and \(\text{Ca}^{++}\) metabolism.

**Transgenic Papaya Plants Via Microprojectile Bombardment**\(^{12}\)

M. M. M. Fitch\(^{13}\)

The objective of our research is to produce transgenic papaya plants expressing the coat protein gene of papaya ringspot virus (PRV). Transgenic plants expressing viral coat protein genes have been shown to be protected from severe virus infection symptoms. Transgenic papaya plants were regenerated from two types of embryogenic tissues after DNA delivery via microprojectiles. Plasmid pGA482GG constructs containing chimeric genes for NPTII, GUS, and PRV coat protein were delivered into apical domes of 90- to 105-day-old pretreated zygotic embryos and into somatic embryos and calli of papaya. After particle bombardment, tissues were subcultured monthly to media containing the selective antibiotic for NPTII, 150 mg l\(^{-1}\) kanamycin, and after 4 months, somatic embryo isolates with apparent kanamycin resistance were observed. Twenty-five isolates were recovered from 57 bombarded cultures. Ten of the isolates were assayed for NPTII expression and all were positive. Five of these were also GUS positive. Four of the NPTII-positive cultures have thus far produced green shoots. Of the two types of tissues bombarded with DNA, the zygotic embryos have yielded larger numbers of transgenic plants and somatic embryos. Thirteen of the 25 bombarded zygotic embryo cultures yielded 19 individual isolates, while only four of the 32 somatic embryo cultures yielded six individual isolates. Thus, transgenic papaya plants have been recovered from microprojectile-bombed tissues, and about 50% of the pretreated zygotic embryo cultures produced at least one transgenic somatic embryo line.

**Mechanistic Relationships between Zeaxanthin-dependent and Independent High-energy Dissipation Components in Pea Chloroplasts**\(^{14}\)

Adam M. Gilmore\(^{15}\)

Photosystem II is protected against harmful overexposure to light through thermal energy dissipation (cf. Krause and Behrend, 1986, FEBS. Lett., 200:298–302). Gilmore and Yamamoto (1989, Proc. 8th Int. Congr. Photosynth., II.6.495) previously demonstrated that two thermal dissipation mechanisms exist in pea chloroplasts, one dependent and one independent of the carotenoid zea-
xanthin. The main objective of this study was to determine whether these components were mechanistically related. Both high-energy dissipation ($qE$) and zeaxanthin formation were monitored simultaneously using room-temperature chlorophyll fluorescence and 500 nm absorbance changes, respectively. Both $qE$ components were dependent on the transthylakoid ΔpH. The zeaxanthin component was linearly correlated with zeaxanthin and apparently additive to the non-zeaxanthin component. The two components differed kinetically during initial induction.

The non-zeaxanthin component levels off in about 1 min, and the zeaxanthin component roughly parallels the kinetics of de-epoxidation. If zeaxanthin is formed during an initial light treatment, then $qE$ kinetics in a second light period are increased such that the $qE$ level at 5 min in the first period is reached in only 1 min. Antimycin inhibited both components but had no effect on zeaxanthin formation. This suggests that in addition to ΔpH, both components are dependent on the redox state of the same electron transport component.

**Energetics of a Predator-prey Interaction: Corals and Coral-feeding Fishes**

**DEBORAH J. GOCHFELD**

Territoriality is commonly exhibited by coral-feeding fishes. Various hypotheses have been proposed to account for this behavior. However, before the adaptive significance of feeding territories can be fully understood, energetics of the predator/prey relationship must be quantified. Energetics of the interaction between the coral, *Pocillopora meandrina*, and the territorial coral-feeding damselfish, *Plectroglyphidodon johnstonianus*, were examined to determine (1) the minimum energetic requirement of the predator, and (2) the effect of predation on the productivity of the prey. Coral productivity for colonies exposed (experimental) and not exposed (control) to predation, and fish metabolic rates were determined using respirometry. Predation by an individual *P. johnstonianus* did not have a measurable effect on coral metabolism. Productivity of experimental colonies (0.148 mg C/cm$^2$/day) was not significantly different from that of control colonies (0.126 mg C/cm$^2$/day). Fish required 237 cal/day, while corals produced 0.209 cal/cm$^2$/day. A typical colony of *P. meandrina* did not produce enough energy to sustain a fish; however, territories of two or more colonies should provide sufficient energy. This may explain why *P. johnstonianus* and other coral-feeding fishes often defend territories consisting of several coral colonies.

**Histological Aspects of Protandrous Sex Change in the Anemonefish, *Amphiprion melanopus***

**JOHN R. GODWIN**

Histological aspects of protandrous sex change were studied in *Amphiprion melanopus* to describe the time course and duration of the process and characterize transformation of the gonad at the cellular level. This is part of a larger study describing protandrous hermaphroditism in this species at the histological, endocrine, and behavioral levels. Sex change was stimulated in the field by removal of the dominant female member of mated, site-attached pairs. Sex-changing males were captured and killed at varying times after female
removal (5, 10, 20, 30, 45, and 100 days; \( n = 3-12 \) for various treatments). Gonads were removed, fixed, and processed by standard histological procedures. Gonad reorganization was rapid after the onset of sex change. Structure of the spermatogenic portion of the gonad was radically different by the 10-day point and nearly all spermatogenic tissue was in the mature spermatid stage. At 20 days, spermatogenic tissue was almost absent and the gonad consisted mainly of previtellogenic oocytes. Virtually all gametogenic tissue in the gonad was ovarian by 30 days. Vitellogenesis was first observed at 45 days. Completion of sex change, as indicated by the presence of mature ova, takes between 45 and 100 days. The pattern of change in gametocytes of the primary sex is fundamentally different from that reported in protogynous species. In protogynous change, primary sex gametocytes degenerate and are phagocytized, while in the protandrous \( A. \) melanopus they differentiate into mature spermatids before being replaced by oocytes.

Isolation, Characterization, and Comparison of Actin Genes from Two Fruit Fly Species

MEI HE

Actin genes have been studied in two fruit fly species, \( C. \) capitata and \( D. \) dorsalis. Both of them are important pests in agriculture. Using a putative actin gene (pmed21) that had been previously isolated, a survey of the expression pattern of this gene from \( C. \) capitata was conducted by using northern blot techniques. The expression pattern showed temporal and spatial specificity and most likely it represents the expression of a muscle actin gene. A cDNA subclone (pmedCl) was also isolated by screening a late pupal cDNA library with pmed21 as probe. This cDNA subclone appears to represent the 3' untranslated (UT) region of the gene in pmed21. It hybridizes to only a single sequence in the \( C. \) capitata genome, and it appears to have homology with a fragment present in \( D. \) melanogaster genomic DNA. This implies that there is homology in 3' UT regions across species. Two actin homologous sequences from \( D. \) dorsalis have been isolated and subcloned into puc9, pDdA1, and pDdA5. Restriction maps for both subclones have been worked out. Using the southern blot techniques, cross hybridization between the subclones pmed21, pDdA1, and pDdA5 has been studied. There is homology between all the subclones, but it is most extensive between pmed21 and pDdA5.

Critical Aspects of Lipofuscin Methodologies for the Accurate Determination of Aging in Aquatic Organisms

KEVIN T. HILL

Quantification of lipofuscin (age-related fluorescent metabolites) has recently been proposed as a means of estimating age in aquatic vertebrates and invertebrates. The present study was undertaken to reassess various aspects of current methodologies adopted for quantifying lipofuscin content in postmitotic tissues. Parameters examined included sample storage and handling times, ultrasonication, solvent systems, and exposure to UV
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light. Brain, heart, and skeletal muscle from the teleost *Oreochromis mossambicus* were used as model tissue systems. Fluorescence of tissues and their extracts increased with both storage temperature and handling time. Tissue extract fluorescence showed increases of up to 100% after 1 hr of incubation at room temperature. Sample sonication and exposure to UV light significantly enhanced and altered fluorescence properties. Stability of sample extracts also varied in each solvent system. These results raise questions as to the nature of the fluorescence measured in previous studies. Age-related patterns of lipofuscin accumulation were subsequently examined in brain tissues of known-age *O. mossambicus* using modified procedures. Brain lipofuscin content significantly increased in specimens ranging in age from 1 month to 2.5 yr, suggesting that this method holds promise as a tool for estimation of chronological age in fish and other aquatic wildstock organisms.

### Hemoglobin Isoelectric Focusing for Unstable \( \alpha \)-Thalassemia Protein Variants from Southeast Asians\(^{24} \)

**James H. Ireland\(^{25} \)**

Isoelectric Focusing (IEF) is very useful for separating hemoglobin (Hb) proteins to detect the \( \alpha \-) and \( \beta \)-thalassemias. It has higher sensitivity than other electrophoretic methods and may prove useful for detecting the unstable \( \alpha \)-thalassemia variants prevalent in Hawaii’s large Southeast Asian populations. Triple \( \alpha \)-gene deletions cause Hb H disease; quadruple deletions cause lethal hydrops fetalis, with serious threat of maternal morbidity. Deletions or mutations in one or more of the four normal \( \alpha \)-globin genes produce a proportional rise in the unstable, fast-migrating Hb Bart’s (\( \gamma_4 \)) at birth. Cord blood Hb Bart’s is 0.5–3% in single \( \alpha \) deletions, 5–12% in double deletions, and 25–35% in Hb H disease. Hb H (\( \beta_4 \)) is found in older patients with triple \( \alpha \) deletions. Hb Constant Spring, an \( \alpha_2 \)-gene chain termination mutation, is a very unstable, slow protein that can produce a severe Hb H disease in combination with a double \( \alpha \)-gene deletion. I have found that these unstable proteins were detected by IEF, but were sometimes missed by cellulose acetate electrophoresis (CAE). IEF was much more sensitive than CAE for detecting Hb Constant Spring. These data show that IEF allows for improved diagnostic accuracy. Fetal testing by DNA analysis can be offered for couples at risk to have children with Hb H disease or hydrops fetalis.

### Misbehaving on a Coral Reef: Observations of a Butterflyfish\(^{26} \)

**Darby K. Irons\(^{27} \)**

The chevron butterflyfish, *Chaetodon trifascialis*, is a solitary, territorial, diurnal, coral-

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females often interacted with each other, and juveniles were found singly or in groups of two to six individuals within the territory borders of adults. A grouping behavior (4–30 individuals) was often seen as well as long excursions by an individual from its territory. *Chaetodon trifascialis* exhibited behavioral patterns predicted by foraging theory for an energy maximizer. Territory sizes were inversely related to food density, and feeding rates were inversely related to intruder rates. This species provides the potential for further testing of more complex aspects of optimization theory.

**Drowned Reefs in the Alenuihaha Channel: Evidence for Island Subsidence and Low Stands of Quaternary Sea Level**

**ANTHONY T. JONES**

Four drowned coral reefs on the deep flanks of Hawaii and Maui were discovered as a result of Deep Tow camera surveys and *Pisces V* submersible observations. Samples collected were analyzed for fossil corals. *Porites compressa* was present in a reef at 950 m depth on the flank of Kohala Volcano. *Porites* was found to be the dominant component of the reef structure, suggesting that it was the principal reef frame builder during the Quaternary in Hawaii. Two reefs on the Haleakala slope at depths of 1000 m and 1200 m have been photographed, but no samples have been recovered. On a deeper reef at 1650 m off Haleakala, specimens of *Acropora* were discovered, indicating that this genus was present throughout the high islands during Quaternary. This work has verified previous studies on the Kohala submarine slope and extends the series of known reefs on deeply submerged terraces of Haleakala. Bathymetric profiles and submersible observations suggest that the reefs off Kohala were barrier reefs implying that conditions for reef development may have been more favorable during the late Pleistocene. The likely cause of drowning was a rapid rise in sea level. Chronology of the reefs has not yet been established, but could yield ages for several low stands of sea level during the Quaternary and also serve as a measure of subsidence for the volcanic pedestals underlying the islands of Hawaii and Maui.

**Platelet-derived Growth Factor–Induced Phosphatidylinositol Turnover, Intracellular Calcium Rise, and Protein Kinase C Activation are not Required for DNA Synthesis**

**MARTHA Y. KANEMITSU**

Platelet-derived growth factor (PDGF) is a potent mitogen for mesenchymally derived cells. In response to a wound, PDGF is released from platelets and deposited in the wound during blood coagulation. In association with other growth factors, PDGF stimulates fibroblast proliferation, connective tissue formation, epithelial regeneration, and scar development. PDGF as a mitogen acts as a control to a number of disease states including cancer and atherosclerosis. Interaction of PDGF with its specific cell surface
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receptor results in activation of receptor tyrosine protein kinase (TPK) activity, an increase in phospholipase C–mediated phosphatidylinositol (PI) turnover, the production of several second messengers including inositol 1,4,5-triphosphate and diacylglycerol, an increase in intracellular calcium, internalization and down regulation of the receptor, auto phosphorylation of the receptor, and initiation of DNA synthesis. Current belief dictates that these early events are critical for PDGF-induced DNA synthesis. Genistein, a specific inhibitor of TPK activity, blocks these PDGF-induced events with the exception of receptor down regulation. We have shown that the reversal of the genistein block results in DNA synthesis but not in an increase in phospholipase C–mediated PI turnover, increase in intracellular calcium, or production of second messengers. These results demonstrate that these early events are not required, as previously believed, for PDGF-induced DNA synthesis.

Sexual Reproduction in Hawaiian Acropora

JEAN KENYON

The coral genus Acropora is a dominant feature of Indo-Pacific reefs, yet only three species occur in the Hawaiian Archipelago, restricted to reefs between Kauai and Laysan. Absence of Acropora from the high islands has been linked to lack of sexual reproduction by local populations, which were hypothesized to depend on immigration of larvae from outside the archipelago for recruitment. The sexual reproductive condition of Acropora valida, A. cytherea, and A. humilis was re-investigated between May and July at French Frigate Shoals. Spawning occurred between 1 and 9 July in A. valida colonies occupying the reef flat habitat and between 11 and 27 July at La Perouse Pinnacle, as determined by the sharp disappearance of pigmented oocytes and testes of mature size. Measurements of oocyte diameters from fixed, dissected polyps indicated linear growth for the 6 weeks preceding spawning. Histological sections of fertile polyps confirmed the maturity of gonads before spawning. A single colony of A. humilis spawned between 6 and 14 June. Strong indications of sexually mature colonies of A. cytherea exist, but clear patterns do not emerge. Lunar periodicity of spawning in reef flat A. valida and in A. humilis differs from that reported for these species from other regions. Ideas concerning the expatriate origin of present larval recruits, as well as the absence of Acropora from the high islands of the Hawaiian chain, need to be reevaluated in light of new evidence for sexual reproductive capacity by local populations.

Molecular Evolution in Sea Urchin Mitochondrial DNA

BAILEY D. KESSING

Understanding molecular evolution in organisms is paramount for the use of molecular data in other fields, namely phylogenetics and evolutionary biology. Recent advances in molecular technologies, such as the Polymerase Chain Reaction (PCR), which allows DNA sequences from small discreet regions to
be rapidly determined, has made it particularly important that differences in molecular evolution within a genome be explored. Armed with a proposed phylogeny for sea urchins in the subfamily Strongylocentrotinae, I have explored mtDNA evolution in this group along evolutionary lineages. In addition, because different coding regions were sequenced, variation in sequence evolution from different gene regions in the mitochondrial genome for this group of sea urchins could be examined. Over 900 bases were sequenced from three mitochondrial DNA (mtDNA) protein coding regions in seven species of sea urchins. Analysis of substitutions at codon positions that result in amino acid replacements (replacement substitutions) and substitutions at codon positions that do not result in amino acid changes (silent substitutions) reveals that these two types of substitutions behave quite differently in different coding regions. Replacement substitutions vary greatly between mtDNA coding regions. In addition, replacement substitutions varied by as much as fivefold along lineages in this group of sea urchins. Silent substitutions, however, do not significantly differ between mtDNA coding regions or along lineages. Selection on amino acid sequences apparently plays an important role in replacement substitutions. Replacement substitutions determined by comparing DNA sequences from different mtDNA coding regions (like those obtained with PCR) may, therefore, give different estimates of the phylogenetic relationships of organisms depending on the coding region used. This suggests that silent substitutions would be much more useful in revealing the phylogenetic relationships of organisms than would replacement substitutions.

Patterns of Resource Use and Aggressive Interaction among Three Species of Coral-feeding Butterflyfish (Chaetodontidae)\textsuperscript{36}

RANDALL K. KOSAKI\textsuperscript{37}

At Johnston Atoll, adults of coral-feeding butterflyfishes (Chaetodon trifasciatus, C. multicinctus, C. trifascialis) defend territories that do not overlap intraspecifically but that overlap extensively with those of congeners. Use of food and habitat resources was studied in relation to territorial behavior among these species. Removal experiments were performed to test the hypothesis that the aggressively dominant species, C. trifascialis, limits access to food resources by the other two species. Only C. trifascialis exhibited interspecific territoriality, defending small territories encompassed within the larger territories of the other two species. Chaetodon trifascialis shared greater overlap in diet with C. trifasciatus than with C. multicinctus, and the frequency of aggressive acts by C. trifascialis was higher toward C. trifasciatus than toward C. multicinctus. Chaetodon trifasciatus and C. multicinctus did not exhibit aggression toward C. trifascialis. For all three species, a majority of aggressive behavior was directed toward conspecifics. Removal experiments ($n = 8$) of C. trifascialis resulted in significantly increased feeding rates and changes in the use of space by C. trifasciatus. Similar removals ($n = 6$) of C. trifascialis produced no change in the feeding of C. multicinctus. Reciprocal removals of C. multicinctus and C. trifasciatus had no effect on feeding by C. trifascialis. Vacated territories were always reoccupied by members of the removed species.

\textsuperscript{36}Partially supported by U.S. Army Contract DACA83-84-C-0019. Sponsor: Ernst Reese.
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In Situ Experiments on Response of Deep-sea Macrofauna to Burial Disturbance\textsuperscript{38}

HELMUT KUKERT\textsuperscript{39}

Formation of sediment mounds by deposit-feeding megafauna is known to disturb the macrofaunal community in the bathyal Santa Catalina Basin, in the Southern California Borderland. I created three types of artificial mounds to study the role of burial due to mound production in structuring the community and to elucidate the importance of different modes of recolonization. Using the submersible \textit{Alvin}, artificial mounds were created on the sea floor and sampled at intervals of 4 days to 2 yr. About 50\% of the macrofauna was able to burrow through 4–10 cm of rapidly deposited sediment. Subsurface deposit feeders were the functional group least susceptible to burial. Formation of mounds reduced abundance of the dominant species, suggesting that burial disturbance may play a role in structuring the macrofaunal community by promoting the coexistence of rarer species. Upward burrowing was the major mode of recolonization over a 4-day to 8-week period. The observed 8-week to 2-yr recolonization rates were about three times higher than those estimated in previous deep-sea studies using colonization trays. Two species showed peak abundances at 2-yr time scales, indicating species succession in artificial mounds.

Transfer along a Continuum in Discriminative Learning of Honey bees (\textit{Apis mellifera})\textsuperscript{40}

YOUNGLIM LEE\textsuperscript{41}

In experiments with dogs, Pavlov (1927, \textit{Conditioned Reflexes}, Oxford University Press) found that discrimination between two closely similar stimuli is facilitated by pretraining with less similar stimuli varying in the same dimension. Analogous results have been obtained repeatedly in work with a variety of vertebrate species, and I have found the same results with honey bees. Experimentally naive honey bees are unable to detect the presence of a 5-\textmu l drop of sucrose on a target, but bees trained first to detect a 20-\textmu l drop of sucrose and then a 10-\textmu l drop are able subsequently to detect a 5-\textmu l drop. In honey bees, as in vertebrates, pretraining with less similar stimuli seems to call the animal's attention to the relevant features of the problem. The results are of interest because they add “transfer along a continuum” to the growing list of vertebrate learning phenomena found in honey bees and because of their bearing on the theory of discrimination learning in honey bees.

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Delayed Plumage Maturation in the Hawai‘i ‘Akepa, *Loxops coccineus coccineus* 42

**Jaan Kaimanu Lepson** 43

Bright adult males in sexually dichromatic birds commonly have a duller subadult plumage during their first breeding season, but extreme variability in male plumage in the sexually dichromatic Hawai‘i ‘akepa has puzzled naturalists for over a century. This variability was studied by documenting the plumage and molt over time in 37 individually color-banded males that were recaptured in mist-nets or resighted with binoculars. Adult males have a permanently bright orange plumage that is preceded by two potential breeding seasons in duller subadult plumage. Plumage is grayish and like that of females during the first potential breeding season and is brighter and more like that of males during the second potential breeding season. Breeding by subadult males was found only during the second season. This 2-yr delay in plumage maturation is unparalleled among sexually dichromatic species with biparental care and is associated with extraordinarily high annual survivorship of adults and extremely long pre-breeding sexual competition.

Production and Characterization of Monoclonal Antibodies to GP195, a Prominent Vaccine Candidate for *Plasmodium falciparum* 44

**Christopher P. Locher** 45

Monoclonal antibodies (MAbs) were produced to a major merozoite surface coat precursor protein (gp195) using the *Plasmodium falciparum* Uganda Palo Alto (FUP) isolate. This molecule is a prominent malaria blood stage vaccine candidate. One group of MAbs identified the N-terminal processing fragment of 83 kDa while another group identified the C-terminal processing fragments of 42 kDa and 19 kDa. Immunoprecipitation of pulse-chase metabolically labeled parasites, Western blot, Immuno Fluorescence Assay (IFA), and Enzyme Linked Immunosorbent Assay (ELISA) were employed for characterization of these MAbs. The MAbs that identified the C-terminus were conformationally dependent epitopes in Western blots, which was detectable when saponin-lysed whole parasite antigen was extracted in the presence of 2-mercaptoethanol. These MAbs also identified the processing fragment in newly invaded, ring-stage erythrocytes. The MAbs were purified for inhibition studies of *Plasmodium falciparum* in vitro. Two of the N-terminal MAbs were partially inhibitory at very high concentrations while none of the C-terminal MAbs were inhibitory. The MAbs can thus be used to directly isolate N- and C-terminal processing fragments for direct vaccine studies using *Aotus* monkeys.

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Characterization of a Developmentally Regulated Ectodermal Gene of the Sea Urchin *Tripneustes gratilla*  

**RICHARD LUM**

cDNAs to ectodermal mRNAs that increase in abundance between fertilization and the pluteus stage of the sea urchin *Tripneustes gratilla* were isolated. Northern blots with cDNA probes revealed the presence of three related mRNAs of 5.8 kb, 2.1 kb, and 1.6 kb. The 2.1-kb mRNA is the most abundant and is loaded with ribosomes. A near full-length cDNA to the 2.1-kb mRNA and a 3-kb cDNA from the 5.8-kb mRNA were sequenced. The three mRNAs likely arise from the same gene and result from differential poly-adenylation. Production of the 5.8-kb mRNA may involve the use of another promoter further upstream and/or differential splicing. The near full-length cDNA has an open reading frame encoding 86 amino acids followed by a long untranslated tail of 1.6 kb. The hypothetical translation product contains a 16-amino acid hydrophobic leader followed by a conspicuous stretch of five or six arginines. A search of the protein database revealed no extensive homology to known proteins. Genomic clones spanning 32 kb of the gene locus were isolated. The gene is approximately 12 kb and contains three exons. The transcription initiation site was determined by primer extension, promoter elements, exon/intron splice junctions, and poly-adenylation signals and sites were identified by sequence analysis. The hypothetical protein minus the hydrophobic leader was expressed as a fusion protein in *Escherichia coli* as a means to generate anti-sera to this protein.

A Temporal-Polygyny-Threshold Model and Spawning in the Pomacanthid Angelfish *Centropyge potteri*

**MARVIN LUTNESKY**

Most animals show temporal patterns of reproduction. They breed at certain times of day, month, or year depending upon the scale examined. Environmental conditions during breeding are usually cyclic (e.g., photoperiod, lunar phase, season) and thus relatively predictable. A temporal-polygyny-threshold model (TPTM) postulates how cyclic environments and costs of polygyny together may influence temporal patterns of reproduction in polygynous animals. Major predictions are that (1) females will compete for breeding order on environmentally advantageous times to breed; (2) losers will choose environmentally less advantageous times to breed when the cost in fitness due to polygyny for the advantageous time exceeds the cost in fitness due to changes in the environment for the less advantageous time; and (3) the amount of the environmental cycle utilized for breeding increases as a function of mating-group size. Although not a direct test of the TPTM, spawning data (n = 72 spawns) for *Centropyge potteri* are consistent with its predictions. Females spawned with their male when he
courted other females in his harem ("interception behavior") in a very conservative estimate of 4.2% of the spawns. *Centropyge potteri* was originally described as having a lunar spawning periodicity. Two extremely large harems held in a tidal pond spawned continuously; however, peak spawning occurred during the same lunar phase as the original description.

Isozyme Variation and Genetic Differentiation in Hawaiian *Metrosideros* from Oahu, Hawaii

**ARADHYA K. MILLIKARJUNA**

*Metrosideros polymorpha* (Gaud.) is a widespread and morphologically diverse native tree species in the Hawaiian rainforest ecosystem. However, four additional species have been recognized that have much narrower distribution. In the present study three species were analyzed for isozyme variation using starch gel electrophoresis. They included *M. rugosa*, with three populations; *M. tremuloides*, with two populations; and *M. polymorpha*, with nine populations. Four enzymes coded by six loci were examined. The mean number of alleles per locus ranged from 2.3 to 3.3 with a mean of 2.97, the percentage polymorphic loci ranged from 66.7 to 100 with a mean of 94, and the mean observed heterozygosity ranged from 0.1 to 0.368 with a mean of 0.272 in different populations. There was a deficiency of heterozygotes in all populations when compared to panmictic expectations. Contingency chi-square analysis for homogeneity of allele frequencies indicated differentiation among the 14 populations but not when these were pooled by species. The mean unbiased genetic identity for pairwise comparisons of morphologically distinct species was 0.904, a very high value obtained for congeneric plant populations. Genetic variation within species was much larger than the variation between species (i.e., 76% versus 24%, respectively). Therefore, in spite of distinct morphological differences, the *Metrosideros* species studied are not genetically differentiable through isozyme analysis.

Larval Life History and Population Subdivision within Two Temperate Sea Urchins (*Heliocidaris*)

**W. OWEN McMILLAN**

We characterized restriction site polymorphisms in the mitochondrial DNA (mtDNA) to access the amount and partitioning of mitochondrial diversity within two temperate sea urchins (*Heliocidaris*). The two urchins exhibit different larval life histories, which affect the length of time a larva spends in the plankton before settling (3–6 weeks for *H. tuberculata* versus 3–4 days for *H. erythrogramma*). Ten informative restriction enzymes were used to assay the mitochondrial genome of 29 individuals of *H. tuberculata* from two localities and 62 individuals of *H. erythrogramma* from five geographic localities. In *H. tuberculata*, 11 mitochondrial genotypes were identified. These genotypes

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were shared between distant localities, suggesting high and continuous genetic exchange over extensive areas. In contrast, *H. erythrogramma* demonstrated significant levels of population structure. Thirteen mitochondrial genotypes within two distinct clades were identified. These clades were partitioned geographically, with western Australia urchins differing from both eastern and southern coast urchins by at least nine restriction site changes. Furthermore, significant population differentiation was evident between eastern and southern coast populations and between southern populations separated by Bass Strait. In those cases, there was no striking genetic discontinuity. Instead, genetic subdivision is due to the distribution of genotypes within each area. This partition of genetic variability argues for very recent population differentiation and implicates recent climatic changes as a force in shaping population dynamics of this species.

**Incipient and Subsequent Stages of Community Development on Select Artificial and Living Substrates in a Marine Environment**

**TEENA MICHAEL**

A field experiment was conducted in a lagoonal back reef area to investigate patterns in early community development on specific substrates. Artificial substrates were used to assess the effects of surface relief and surface charge on colonization. Early colonization patterns on the living substrate, *Halophila hawaiiana*, were also assessed. Films were generally observable after 1 day of immersion, as viewed by scanning electron microscopy (SEM). Film development and accretion of other surface components at 3 days and up to 30 days were further observed by SEM and analyzed for relative frequencies, relative abundance, and correlations. Film chemistry of one sample was probed by pyrolysis GC/MS. Contrasting patterns occurred in filming and colonization after 3 days of immersion on artificial substrates. The mildly positive surface is consistently more inhospitable to the commonly observed organisms than strongly positive and negative surfaces. An oxidized surface (strongly negative) hosted a rich community. Convergent community composition after initially dissimilar colonization among all substrates did not occur within 30 days.

**Physical Basis of Direct-interception Feeding by Marine Zooflagellates**

**BRUCE C. MONGER**

Direct-interception feeding by marine zooflagellates is a complex process that can be described in terms of a sum of separate component forces. A general mathematical framework for obtaining estimates of rates of ingestion and clearance (volume cleared time\(^{-1}\) flagellate\(^{-1}\)) from knowledge of hydrodynamic and surface force components has been formulated. Approximate expressions for London-van der Waals, electric double-layer, hydration, and fluid-drag forces were
utilized within this general framework to obtain first-order predictions of zooflagellate clearance rates and associated size dependencies. When standard surface-force parameters and grazer swimming speed were used, clearance rate predictions were generally within a factor of two of observations. Clearance rate, as a function of prey ($R_p$) and grazer radii ($R_p, R_g$), was predicted to be roughly proportional to $R_p^{0.8}R_g^{0.7}$, which is incompatible with previous model predictions but generally agrees with recent observations. This modeling approach represents a powerful tool for establishing hypotheses concerning the physical mechanisms underlying direct-interception feeding by marine zooflagellates.

**Enigmonia aenigmatica: An Enigmatic Molluscan Chameleon**

**SHAUN M. MOSS**

The ability of molluscs to change shell color in response to environmental heterogeneity is rare and is previously unknown in bivalves. The anomiid bivalve *Enigmonia aenigmatica* inhabits Southeast Asian mangrove communities and exhibits two discrete colors, cream and purple-brown, within a single population. Previous researchers have regarded these varieties as either two distinct species or as conspecific color morphs. Studies were conducted to (1) determine if shell color is associated with a particular substrate type, and (2) determine if *E. aenigmatica* can change shell color when transplanted from one substrate type to another. Results of field transects indicate that shell color is not independent of substrate type. Cream individuals were only found on green *Avicennia* spp. leaves, whereas 94% of purple-brown individuals were attached to purple-brown *Avicennia* spp. trunks and stems. In laboratory transplant studies, 71% of cream *E. aenigmatica* exhibited discrete purple-brown pigmentation bands along their shell margins after 3 weeks when transplanted from *Avicennia* spp. leaves to pieces of *Avicennia* spp. trunk. A control group, consisting of cream *E. aenigmatica* attached to leaves of *Avicennia* spp. saplings, exhibited no color change after 6 weeks. *Enigmonia aenigmatica* exhibits shell color plasticity by changing its color to match that of its background. This ability may confer an adaptive advantage by providing protection from visual predators.

**Effects of Nutrition, Glucose, and Amino Acids on Prolactin (PRL) and Growth Hormone (GH) Release in vitro from the Pituitary of the Tilapia, Oreochromis mossambicus**

**BUEL D. RODGERS**

Prolactin and GH play roles in development, reproduction, osmoregulation, and metabolism. Our studies were aimed at determining whether PRL and GH cells were sensitive to nutritional status, medium d-glucose, and amino acids. Two groups of male fish (50 to 60 g) were starved or fed twice daily for 2
weeks. The rostral pars distalis (RPD), containing a nearly homogeneous mass of PRL cells, was then incubated for 18–20 hr in either hyposmotic (HO: 300 mOsmolal) or hyperosmotic medium (HR: 355 mOsmolal). The proximal pars distalis (PPD), containing GH cells, was incubated for 18–20 hr in isosmotic (320) medium. Starvation reduced PRL release in vitro. In pairwise comparisons, this effect was significant only in HO, in which PRL release is normally stimulated, but not in HR, in which PRL is normally inhibited. Starvation substantially increased GH release. Also, PRL release was unresponsive to variations in medium d-glucose, although GH release varied inversely with concentrations ranging from 12.5 mg/dl to 100 mg/dl and with medium MEM concentrations from 0.25 ml/dl to 4 ml/dl. PRL release also varied inversely with these MEM concentrations. These findings suggest that PRL cell activity may be sensitive to nutritional status, but it is unlikely that this is mediated by changes in blood glucose, although PRL cell activity may be sensitive to amino acids. The augmentation of GH release by reduced glucose and amino acids in vitro may explain the high spontaneous release of GH observed in PPD taken from starved tilapia.

17-Methyltestosterone (MT) and Acclimation Salinity Effects on Nutrient Absorption by the Intestine of the Tilapia, *Oreochromis mossambicus*62

Benny Ron63

Use of growth promoters is becoming an increasingly important aspect of aquaculture. Although studies suggest that they increase intestinal nutrient absorption in cold-water fishes, little is known about their effects in warm-water species. In this study, effects of MT on proline and glucose absorption by the anterior intestine of tilapia reared in fresh water (FW) and in seawater (SW) were examined. Nutrient absorption was measured using the everted sleeve technique. Segments of the anterior intestine are removed and everted to turn the luminal, absorptive surface outward. The surface is then exposed to radiolabeled proline or glucose for 4 min to quantify the uptake rate of the nutrient. Active (Na+ -dependent) uptake is distinguished from passive uptake by use of a metabolically inert marker or by paired incubations with and without Na+. Treatment with MT reduced passive, length-specific proline uptake (uptake per centimeter of intestine) in SW animals and passive, weight-specific proline uptake (uptake per milligram of intestine) in FW animals. By contrast, MT treatment increased active, length-specific glucose uptake in intestines from FW animals. Active, length-specific proline uptake was increased and active, weight-specific glucose uptake was decreased in intestine from SW animals compared with those from FW animals. Changes in length-specific uptake are believed to represent differences in the diameter and, thus, surface area of the lumen. Changes in weight-specific uptake, however, reflect differences in the intrinsic transport properties of the intestine.

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Intertidal zones are heterogeneous environments, demonstrating great spatial and temporal variability. Dramatic physiochemical gradients are manifest in the residual pools along the intertidal zones of Hawaii's shores and contribute significantly to community structure, life history tactics, and interactions of the inhabiting biota. Distribution and growth rates were examined for the brown goby, *Bathygobius fuscus*, along the tidal flats of Sandy Beach (Oahu, Hawaii). Often, the most ideal habitat space was occupied by competitively superior adults that could cannibalize or exclude new recruits and small juveniles. Suboptimum habitat selection by smaller individuals imposes the necessity to acclimate to less suitable and highly fluctuating environments. Ability to respond appropriately to local environmental conditions while optimizing growth rates may be critical for recruitment success and survival of *B. fuscus*. Growth rates during critical life-history events were estimated using otolith microincrement enumeration techniques. Growth of *B. fuscus* is most likely governed by a trade-off between selective pressures for fast growth and the energetic costs associated with predator avoidance and acclimation in an unpredictable environment.

This study is part of an ongoing baseline research project studying the biology of *Awaous stamineus*. Biological information is needed on this species to formulate and implement management programs for Hawaiian rivers and the goby fishery. Little is currently known about the reproductive biology of *A. stamineus*; this field study has produced preliminary results on this subject. Gonadal maturation and its relationship to fish size and spawning season was studied from June 1989 through March 1990 in fish ranging in standard length from 56 mm (3.5 g) to 232 mm (149 g). Preliminary data showed changes in the gonadosomatic index of females, which indicated increased reproductive activity from August through December. The standard length at first reproduction for both sexes was ca. 75 mm. Surveillance of spawning in Hanalei and Wainiha rivers provided some details of behavior and use of the substrate for nesting during a brief spawning event in October. The Wainiha spawning was concentrated at a different location and involved fewer fish than a spawning recorded in Wainiha in 1988. Spawning in Lumahai River was on a smaller scale but was more prolonged than in the other rivers and was concentrated near the river mouth in contrast to the more upstream spawning sites in the other rivers.
Many fishes nest in groups, probably to decrease predation of offspring. Paternal care of offspring is exhibited in these fishes. Given the potential advantages of group nesting, do females spawn preferentially in larger groups and is male size (total length) related to reproductive success (RS)? Colony size variation exhibited by the damselfish (Pomacentridae: *Abudefduf abdominalis*) allow assessment of the effect of colony size on male RS. Previous studies have shown that males nesting in large colonies (> 7 nests) receive more clutches and suffer less egg predation than males nesting in small colonies. However, in these studies male and nest site characteristics were confounded. To determine the relative contribution of male size and nest site characteristics to RS, a male removal experiment was performed. Cement slabs controlled for nest substratum quality. These data show that current RS is directly related to previous RS of the male, independent of the nest site. Past and current nest survival is directly related to current RS. Male length is weakly related to current RS. The results suggest that important criteria of female choice are both the ability of males to defend against egg predators and colony size.

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Scanning Electron Microscopy of Radular Structure in the Neritidae

Catherine Craine Unabia

The molluscan gastropod family Neritidae, an ancient offshoot from the main line of gastropod evolution, developed numerous unique characteristics during radiations into marine, intertidal, freshwater, and semiterrestrial habitats. To explore phylogenetic relationships in this group, radulae from representatives of the eight living neritid genera were compared using scanning electron microscopy (SEM). A typical radular plan was identified with consistent tooth numbers, shapes, and interactions between neighboring teeth within and between rows found in all except *Neritilia* (subfamily Neritiliinae Baker, 1923). In this divergent taxon homologies between teeth are difficult to determine. Differences in reproductive anatomy support the suggestion that the genus *Neritilia* be removed from the family Neritidae. Great similarity was seen between radulae from *Nerita, Neritina, Neritodryas, Theodoxus, Clithon,* and *Septaria,* confirming their placement together in subfamily Neritinae Baker, 1923. Although the radulae of *Smaragdia* follow the basic neritid pattern, including interactions between teeth, differences in tooth structure place the genus in subfamily Smaragdiinae Baker, 1923. However, their radular differences may be explained by a unique feeding specialization on the cytoplasm of seagrass leaf cells, punctured by the prominent cusps of the modified outer lateral teeth. Thus separation from the other nerites may not be warranted.

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Molecular Analysis of Bean Arcelin Gene(s) Encoding an Insect-resistant Protein\textsuperscript{72}

MING-LI WANG\textsuperscript{73}

Arcelin, a seed storage protein originally found in wild beans (\textit{Phaseolus vulgaris}), has insecticidal activity on an important bean bruchid pest, \textit{Zabrotes subfasciatus}. There is ca. 80\% homology between the nucleotide sequences of the cDNA's encoding arcelin-I and bean seed lectin (Osborn et al., 1988, Science 240: 207–210). We have constructed a genomic library for bean line SG3+7, containing arcelin-I protein, and isolated 15 clones by using the arcelin-I cDNA as a probe. A 19 base oligonucleotide based on the sequence at the region having the least homology (50\%) between arcelin and lectin genes was synthesized and used to identify the arcelin clones. Among the 15 potential clones, only 7 hybridized to the synthetic probe, including \( \lambda \)Pv222. A 2.3-kb EcoRI fragment from \( \lambda \)Pv222 was subcloned into pVZ-1 and sequenced. The coding sequence of this arcelin-I gene shows perfect match to the cDNA. Similar to lectin genes, arcelin gene is intronless, and partial sequence from the 5' upstream region shows 65\% homology to the corresponding region in the lectin gene. A 23-bp sequence (GCCACCT-AG-T-C-CCTCTTC) in the lectin promoter region appears twice in the arcelin-I gene.

Dynamics of Sexual Selection: Male Mating Success and Female Fitness in a Laboratory Population of the Mediterranean Fruit Fly\textsuperscript{74}

TIMOTHY S. WHITTIER\textsuperscript{75}

The theory of sexual selection is well established, yet data measuring the effect mate choice has on fitness are sorely lacking. The purpose of this study was to provide data concerning effects of mate choice in an organism that exhibits strong sexual selection. Although females of the Mediterranean fruit fly are thought to be monogamous, males show strong differential mating success (i.e., sexual selection via female choice), with a mating range of 0–11 matings in a competitive environment. Even though a male may mate many times, a male's first mating was shown to produce up to one-half of the offspring sired. Females that mated with virgin males produced significantly more offspring than females that mated with nonvirgin males. The period of recovery for the male partner showed no relationship to the number of offspring produced by his mate. In addition, females mating with virgin males also showed significantly increased longevity compared to females that mated with nonvirgin males. Possible evolutionary explanations were explored and the possible economic effects such data may have on current sterile insect release programs were discussed.

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