JEB Classics is an occasional column, featuring historic publications from *The Journal of Experimental Biology*. These articles, written by modern experts in the field, discuss each classic paper's impact on the field of biology and their own work. A PDF of the original paper accompanies each article, and can be found on the journal's website as supplemental data.

FOUNDATIONS OF GREGARIOUSNESS IN BARNACLES


[584]

LABORATORY EXPERIMENTS ON GREGARIOUSNESS DURING SETTING IN *BALANUS BALANOIDES* AND OTHER BARNACLES

By E. W. KNIGHT-JONES

Marine Biology Station, University College of North Wales, Bangor

(*Received 27 March 1953*)

(With Plate 16)

1. INTRODUCTION

Cirripede larvae must become widely dispersed during their long planktonic life, yet the adults of many species are found exclusively in restricted habitats. Some are epizoic and others are parasitic on specific hosts, which the cypris larvae must therefore seek out and recognize, whilst cyprids of many Lepadidae and Rhizocephala are known to seek out adults of their own species, to which they attach themselves as dwarf or complemental males. Burton (1949) pointed out that cyprids of *Balanus balanoides* (L.) seem to be gregarious during setting, and field experiments indicated similar behaviour in *Elminius modestus* Darwin (Knight-Jones & Stevenson, 1950). The following experiments have established that these common littoral forms are able to recognize their own species, and have thrown some light on the mechanism of recognition.

2. METHODS

Cyprids of *Balanus balanoides* were collected near Bangor during April and May, by picking up *Fucus* from shallow water and rinsing the fronds in wide-mouthed jars (breffits), which contained about 2 l. of sea water. Large numbers of cyprids were dislodged from temporary attachment to the fronds, and were later pipetted away to clean vessels, taking advantage of their positive phototaxis.

Cyprids of *Balanus crenatus* Bruguière and *Elminius modestus* were obtained from plankton hauls. They could be readily separated from the rest of the catch, as they became trapped in the surface film when left undisturbed in shallow dishes. Apparently the cyprid cuticle is more strongly hydrophobic in these species than in *Balanus balanoides*.

Experiments were carried out in uniformly illuminated glass dishes, containing 250 ml. of sea water, advantage being taken of the fact that glass is unfavourably smooth for the setting of barnacles, especially of *B. balanoides* (Runnström, 1925; Barnes, Crisp & Powell, 1951). Records were made of the numbers setting on surfaces which had been treated in different ways. Cyprids were usually active, alternately swimming and crawling. Their exploratory habits enabled them to choose between two surfaces presented to them in the same dish. A cyprid which

Robert J. Toonen writes about E. W. Knight-Jones' classic 1953 publication on gregariousness during settling in barnacles.

One of the major thrusts of marine biologists through the 1950s and into the 1960s was to examine the role of settlement behavior of planktonic larvae in contributing to the distribution and abundance of adult marine invertebrates. This effort was the birth of both supply-side ecology and the first attempts to isolate and characterize the specific substratum-bound substances to which larvae respond at the time of settlement. E. W. Knight-Jones was among the leaders in this area, along with a handful of others (reviewed by Young, 1990), and his work continues to be widely emulated and expanded upon by researchers in the field of larval ecology today. A literature survey by Pechenik et al. (2001) indicates that relatively few papers continue to be cited more than 50 years after they are published, and fewer still are cited more often now than when they were first published. E. W. Knight-Jones' (1953) article published in the *Journal of Experimental Biology* is among those exceptions that continue to be widely cited in the modern literature. In fact, this paper has been cited 196 times since 1990, and nearly a half-century after publication this paper continues to be cited in such prominent journals as *Proceedings of the Royal Society of London Series B*, *Ecological Monographs*, *Ecology*, *Oecologia*, *Evolution*, *Peptides*, *Aquatic Microbial Ecology* and *Marine Ecology Progress Series*.

Knight-Jones' paper sought to understand the role of settling behavior of larval barnacles on establishing the aggregated distribution of adults. Approximately 80% of marine organisms (about 90,000 species), both vertebrate and invertebrate, have a biphasic life-cycle and produce planktonic larvae, which spend some variable amount of time (ranging from minutes to months) developing in the water column before settling and metamorphosing into the adult life-form (Thorson, 1950). The choice of settlement site is important because the animal will remain here for the rest of its adult life. Knight-Jones tested the attractiveness of a variety of substrates to larval barnacles at the time of settlement. Through a series of laboratory settlement assays, he established that barnacle larvae recognize cues specifically associated with adult barnacles, are attracted to these cues, and therefore settle near conspecifics. Further, this paper was among the first to attempt to characterize the chemical nature of a settlement cue or metamorphic inducer for the larvae of marine invertebrates (for a recent review, see Pawlik, 1992). Knight-Jones' paper presented only qualitative observations of settlement preferences. However, his results are generally sufficiently striking that the absence of statistics does not impede interpretation of the results, and this paper remains among the more thorough of single papers on the settling behavior and nature of the settlement cue for any marine organism published to date. The paper is most widely cited for two different aspects: (1) it presents convincing evidence that larval barnacles are gregarious and active in their settlement choices, and (2) it reveals that the contact-dependent cue to which larvae respond appears to be a cuticular protein. However, there are many additional nuggets in this paper that continue to make it a worthwhile read for larval biologists today.

Knight-Jones showed, for example, that cyprid larvae, the specialized stage of barnacle larval development that is competent to settle, are capable of prolonging their planktonic lives and delaying metamorphosis into juveniles. The capacity to delay metamorphosis is considered adaptive because it increases the likelihood of locating suitable habitats and may promote genetic exchange among populations (reviewed by Pechenik, 1990). Knight-Jones' paper was certainly not the first to document a capacity for competent larvae to delay metamorphosis, but it was one of the early papers that document a cost to that delay. Although Knight-Jones attributed the cost solely to larval encumbrance by bacterial growth, he documented that juveniles from delayed settlers were capable of metamorphosing at a reduced size, and that larvae which delayed by more than 2 weeks became sluggish and were frequently unable to settle.

The paper also presented one of the first comprehensive surveys of potential settlement cues and experimental treatments to determine the chemical nature of the substratum-derived cue (reviewed by Rittschof and Cohen, 2004). Through serial exposure of the putative cue (the bases of conspecific barnacles) to heat, solvents, acids and alkalis, oxidizing agents and, finally, reagents that react with proteins, Knight-Jones concluded that the cue was most likely a quinone-tanned protein from the epicuticle of settled barnacles (for a recent review, see Clare and Matsumura, 2000). He went on to demonstrate that settlement in response to conspecific barnacles was highest, but response to other species of barnacles was still higher than settlement on uninhabited surfaces (also reported by several recent workers, such as Matsumura et al., 2000). Knight-Jones also points out that secondary cues (additional cues beyond simply the presence of the epicuticular protein) are important to barnacle recruitment decisions because larvae 'sensitized' by physical contact with conspecifics would settle on nearby uninhabited rough granite rather than smooth glass surfaces inhabited by barnacles. The potential disadvantage of attaching to a perfectly smooth surface, such as plate glass, is obvious for an organism living in the pounding surf. The specific ranking of settlement cues used by barnacle larvae was later explored in detail by Wethey (1986).

Finally, there is an observation, mentioned several times in Knight-Jones' paper, that "*isolated bare surfaces collect abnormally undiscriminating pioneer settlers, which are soon followed by gregarious individuals.*" This observation, apparently viewed as an aberrant behavior by Knight-Jones, was later followed up in a number of papers (e.g. Krug, 2001; Toonen and Pawlik, 1994) that documented similar behavior among the larvae of other species, and argue that this strategy is a form of bet-hedging (reducing the risk of reproductive failure by distributing offspring among a variety of habitats) or a dispersal polymorphism (production of two or more larval types with differential proclivity to disperse and settle). Such variation in settlement preferences among individual larvae should increase individual fitness for marine invertebrates with planktonic larvae (reviewed by Toonen and Pawlik, 2001). Knight-Jones argued that the level of discrimination shown during larval settlement should be subject to individual variation, an idea that was largely lost to the field until rekindled by Raimondi and Keough (1990), and later demonstrated experimentally (e.g. Gibson, 1995; Krug, 2001; Toonen and Pawlik, 1994).

With the volume of publications today, no one can read all the papers coming out each year in any particular field. Researchers are routinely turning to electronic databases to search the massive volume of literature in their field, but even the best of these databases contain only about 30 years of citations. Given the ease with which it is possible to search electronic databases today, it is not surprising that relatively few of the papers published before the age of computers are commonly cited in the current literature (Pechenik et al., 2001). Thus, I applaud the concept of the JEB Classics, and am honored to be part of the effort to reintroduce classic papers from before the age of PDFs into the electronic databases of today. E. W. Knight-Jones' classic JEB paper has provided a great deal of inspiration for researchers in the field of larval ecology, and continues to be cited widely today. It was a pivotal paper in formulating my early research interests, and I hope that making it easily accessible to future generations of larval biologists will continue to provide similar stimulation.

This is contribution #1193 from the Hawaii Institute of Marine Biology and SOEST #6544.

A PDF file of the original paper can be accessed online: http://jeb.biologists.org/cgi/content/full/208/10/1773/DC1

10.1242/jeb.01628

Robert J. Toonen
University of Hawaii at Manoa
toonen@hawaii.edu

References

Clare, A. S. and Matsumura, K. (2000). Nature and perception of barnacle settlement pheromones. *Biofouling* **15**, 57-71.
Gibson, G. (1995). Why be choosy? Temporal changes in larval sensitivity to several naturally-occurring metamorphic inducers in the opisthobranch *Haminaea callidegenita*. *J. Exp. Mar. Biol. Ecol.* **194**, 9-24.
Knight-Jones, E. W. (1953). Laboratory experiments on gregariousness during setting in *Balanus balanoides* and other barnacles. *J. Exp. Biol.* **30**, 584-599.
Krug, P. J. (2001). Bet-hedging dispersal strategy of a specialist marine herbivore: a settlement dimorphism among sibling larvae of *Alderia modesta*. *Mar. Ecol. Progr. Ser.* **213**, 177-192.
Matsumura, K., Hills, J. M., Thomason, P. O., Thomason, J. C. and Clare, A. S. (2000). Discrimination at settlement in barnacles: Laboratory and field experiments on settlement behaviour in response to settlement-inducing protein complexes. *Biofouling* **16**, 181-190.
Pawlik, J. R. (1992). Chemical ecology of the settlement of benthic marine invertebrates. *Oceanograph. Mar. Biol. Annu. Revs* **30**, 273-335.
Pechenik, J. A. (1990). Delayed metamorphosis by larvae of benthic marine invertebrates does it occur? Is there a price to pay. *Ophelia* **32**, 63-94.
Pechenik, J. A., Reed, J. M. and Russ, M. (2001). Should auld acquaintance be forgot: Possible influence of computer databases on citation patterns in the biological literature. *Bioscience* **51**, 583-588.
Raimondi, P. T. and Keough, M. J. (1990). Behavioral variability in marine larvae. *Aust. J. Ecol.* **15**, 427-438.
Rittschof, D. and Cohen, J. H. (2004). Crustacean peptide and peptide-like pheromones and kairomones. *Peptides* **25**, 1503-1516.
Thorson, G. (1950). Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.* **25**, 1-45.
Toonen, R. J. and Pawlik, J. R. (1994). Foundations of gregariousness. *Nature* **370**, 511-512.
Toonen, R. J. and Pawlik, J. R. (2001). Foundations of gregariousness: A dispersal polymorphism among the larvae of a marine invertebrate. *Evolution* **55**, 2439-2454.
Wethey, D. S. (1986). Ranking of settlement cues by barnacle larvae: Influence of surface contour. *Bull. Mar. Sci.* **39**, 393-400.
Young, C. M. (1990). Larval ecology of marine invertebrates a sesquicentennial history. *Ophelia* **32**, 1-48.