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A productive friendship – my work in ant cytogenetics with Ross H. Crozier

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Abstract

Three myrmecologists (R.H. Crozier, R.W. Taylor and H.T. Imai) with different academic backgrounds and interests met in Australia in 1975. They later established an air-drying technique for ant chromosome observations and surveyed hundreds of Australian ant species centred around the Jack jumper *Myrmecia pilosula*, and proposed the so-called "Minimum Interaction Theory" (a new theory for chromosome evolution). This is an account of their work together.

Key words: Formicidae, *Myrmecia pilosula*, cytogenetics, population genetics, taxonomy, chromosome evolution, review.

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Prologue

On 8 October 2009 I received from the editors of Myrmecological News a manuscript on ant karyotype evolution by P. Lorite & T. Palomeque (I waived anonymity as referee). It included the following paragraph:

"In ant cytogenetics, the groundbreaking work was performed by Hirotami T. Imai and Rossiter H. Crozier, who, with coworkers, analysed the majority of ant karyotypes. Crozier published a monograph in 1975, reviewing Hymenoptera cytogenetics (CROZIER 1975). … In 1977, IMAI & al. published a paper on Australian ants in which karyotypes of a total of 150 species were presented. … These works are important not only for the large number of species analysed but also because chromosome polymorphism in ants is analysed and a hypothesis for chromosome evolution in this group, the "minimum-interaction theory", is given for the first time. This theory seeks to establish the possible mechanisms governing ant karyotype evolution (IMAI & al. 1986, 1988a)" (LORITE & PALOMEQUE 2010).

One month later (on 12 November), I was stunned by two e-mail messages, one from the Myrmecological News editors, the other from my Australian colleague and friend Dr. Robert W. Taylor, informing me that Ross H. Crozier had passed away that day. During a long silence, I was struck with vivid memories of the past collaborative work with my dear friend, which I wish now to recall.

A fateful encounter

Our longstanding partnership began in September 1965, when, at the suggestion of Professor William L. Brown Jr. of Cornell University, Ross sent me a set of beautiful micrographs of ant chromosomes (23 September). He was then shortly to obtain a M.Sc. from the University of Melbourne (Australia), where his supervisor was the world famous cytogeneticist Professor Michael J.D. White. Immediately afterwards, Ross moved to Brown's laboratory as a Ph.D. student. I greatly appreciated a letter from Professor Brown on 27 September 1968, suggesting that "It would be nice to have you here while Crozier is still here; he should be finishing his Ph.D. next summer (1969)." Ross published his first paper of ant chromosomes in 1968 (CROZIER 1968), the first of more than a dozen contributions prior to 1973.

I began to work in cytogenetics slightly earlier than Ross. I was a graduate student of the Tokyo Kyoiku University (Japan), and was introduced to cytogenetics at The Japanese National Institute of Genetics (NIG) in 1962. I published a small paper on ant chromosomes in 1966 under the guidance of Professor Brown (IMAI 1966). A D.Sc. followed in 1967, and the resulting thesis was published as IMAI (1969) with support from Professor Edward O. Wilson (Harvard University). I became an employee of NIG in the same year, appointed as a research associate in mammalian cytogenetics. Ant cytogenetics remained my avocation and private hobby.

Several years later, Ross wrote me (24 September 1971) that "I am preparing to write a small monograph on hymenopteran chromosomes." He asked: "I would be most grateful if you would send me a few photographs for possible inclusion in the monograph." We had by then developed chromosome observation techniques independently using quite different methodology. He had devised an air-drying technique, while my method was based on squash preparations. Although we both obtained good mitotic chromosome preparations in those days, our techniques each had advantages and disadvantages. HAUSCHTECK (1961, 1962) had correctly reported ant chromosome numbers prior to us. However, because our methods had potential to illustrate not only chromosome numbers but also chromosome morphology (i.e., karyotypes) in detail, we became effectively the pioneers of modern ant cytogenetics.

Two years later (30 October 1973), I asked Ross if it would be possible for me to visit his lab for a couple of years. He was then already a rising ant cytogeneticist (as an assistant professor at the University of Georgia, USA). Our plan proceeded smoothly, and I obtained a grant from the Japanese government to visit him as an exchange visitor, with plans to start on 1 September 1974.

However, on 29 May 1974, Ross informed me that "I have been offered a position as Lecturer in the School of Zoology at the University of New South Wales, Sydney, Australia, and I have accepted this offer. … As I will be taking up the position at the end of this year or the beginning of 1975, it is obviously not practical for you still to join me here in Georgia." He proposed work at Brown's Cornell laboratory or that I could join him at Sydney after his arrival. I chose the latter, because we both wished collectively to improve our chromosome observation techniques by cooperative work. I was also influenced by his persuasive (and very true) words that "the Australian ant fauna is truly immense and diverse (about 2,000 spp.)."

Establishment of an improved air-drying technique for ant chromosome observation

Early in the long-awaited year 1975 (4 February), Ross sent me a last message: "Well, we are now in Sydney and starting to settle in. By the time you and your family arrive, we should no longer be totally unfamiliar with things around here!" A month later (2 March), my family and I arrived at last at Sydney airport, where we met a calm and intelligent gentleman, who was surely the Ross I expected. He was four years younger than me, but he was a really multi-talented scientist, because he had profound knowledge not only of cytogenetics but also of biochemistry, molecular genetics and even population genetics! Moreover, he already possessed a reputation as a leading scientist. Although I was very active and talkative in Japan, my poor English compelled me to be a typically silent Japanese scientist while I was in Australia. As a result, our personal communications were often a little confused.

However, we found great satisfactions in our cooperative activities. We first demonstrated our chromosome observation techniques to each other, and within a few days, we had established an improved air-drying technique which skilfully incorporated my squash technique. We soon attempted field work to test the new method. From this period, I have a cherished snapshot of Ross collecting ants in the field (Fig. 1). He did confess however, that he preferred desk work over field work, which suited him well (Fig. 2).

Our new method was not only easy but also very powerful. For example, we were able to make chromosome preparations within an hour in the field, working right beside subject ant nests (Fig. 3). Even more surprising was the fact that the chromosomes obtained were beautifully slender, often displaying excellent C-banding without other treatment. We were able to survey the chromosomes of 105 Australian ant species before the end of 1975. I worked so hard that Ross finally told me apologetically "Hirotami, the budget for our work is exhausted already!"

In spite of such progress, we faced another serious problem – we did not know the scientific names of the ants we had examined! This was fortunately solved by cooperation with Robert (Bob) Taylor, a former New Zealander, who had obtained his Ph.D. under the supervision of Professor Wilson at Harvard, and was at that time the chief taxonomist and curator of ants at The Australian National Insect Collection, Australian Commonwealth Scientific and Research Organization (CSIRO), Canberra. Thanks to Taylor, we had successfully avoided what is now referred to the "Taxonomic Impediment" (a term first introduced by him).

Fig. 1: Ross H. Crozier (centre) collecting ants in the field with P.J.M. Greenslade (front right) and R.B. Halliday (back right) (near Morgan, South Australia, 1975). Photographed by H.T. Imai.

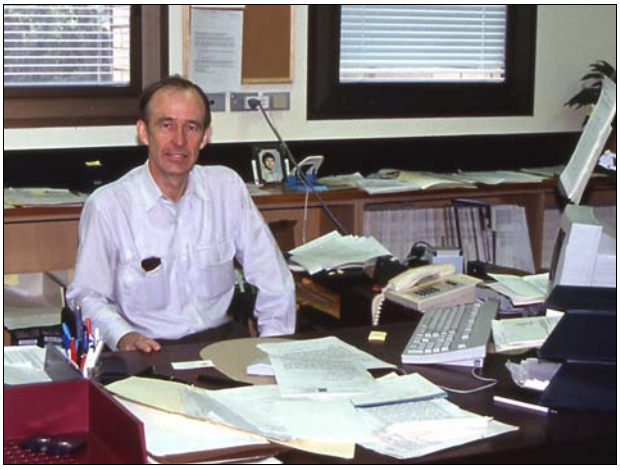

Fig. 2: Ross H. Crozier was always an effective desk worker (LaTrove University, 1991). Photographed by H.T. Imai.

Fig. 3: The author making chromosome preparations in the field (Kudgee near Broken Hill, New South Wales, 1975). Photographed by Ross H. Crozier.

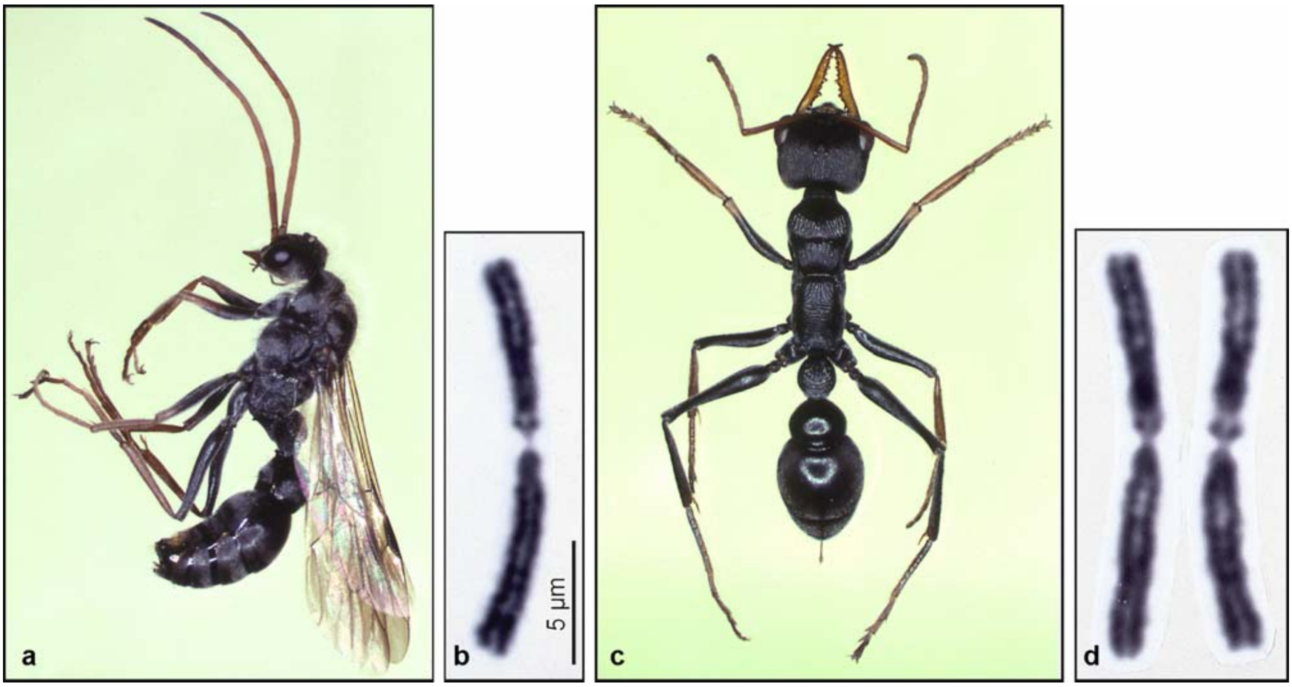

Fig. 4: *Myrmecia croslandi*. (a, b) A male with n = 1; (c, d) a worker with 2n = 2. Photographed by H.T. Imai & M. Kubota.

We then began actively to prepare a monumental paper on Australian ant chromosomes. The first draft was sent to Ross on 16 March 1976, immediately after I returned to Japan. It was really voluminous (53 text pages with 24 figures and 10 pages of chromosome data lists). Ross returned a revised manuscript on 9 May, in which traces of his conscientious effort were evident throughout. Finally, he sent on 9 June copies of the final version to the famous cytogeneticist Professor Bernard John (Australian National University, ANU, Canberra) and his respected former supervisor Professor White. Ross called White a few weeks later enquiring about the manuscript, and received positive feedback that the work was "impressive in size and in scope." This was an epoch-making event for us, because White was the world authority on the "Fusion Hypothesis" (WHITE 1973), and our data supported the opposing heterodox "Fission Hypothesis"! In fact, we could foresee White's tacit approval, because when we first visited Taylor in Canberra, Ross delivered a seminar about our studies at ANU, and White attended.

Almost simultaneously, Ross received a short letter from Professor John on 6 July, and informed me immediately that "he finds our paper "important and interesting", but wants to talk it over with me, rather than sending a set of detailed comments. … p.s. I suspect that John may have some hard comments, but I am optimistic that he will support us overall." Ross' expectation was correct, because the draft was revised several times by him and John before the end of August.

I received a telegram from Ross on 16 October 1976. It read: "JOHN ACCEPTED MANUSCRIPT WITH MINOR REWORDING, ROSS". My ever-calm friend seemed very excited at the time. Anyway, he sent me a more detailed letter on the same day. He said "By now you will have received my telex saying that John has accepted our paper. He has transmitted it to Bauer (the editor of Chromosoma) with only very minor wording changes and corrections. Proof should be sent in about three months, and the paper appears some three months or so after that. … Prof. John urged that I be the one to whom proof is sent." Ross continued "John's rationale was that my proximity to him, and my (marginally) better command of English, combine to make this arrangement worthwhile. I hope you will agree with this procedure, and assure you that I will deal with the proofs with all due speed!" I note that Ross' use of the word "marginally" is a nice example of his gentle sense of humour. Of course, there was no problem on my part, because I wished earnestly to see our joint Magnum Opus (as Ross called it) published in Chromosoma! It was published as IMAI, CROZIER & TAYLOR (1977).

Large scale chromosome surveys centred on the *Myrmecia pilosula* species complex

In parallel with our ant work, I had been working on a theoretical approach for chromosome evolution with Drs. Takeo Maruyama and Takashi Gojobori (population geneticists in NIG). Ross joined our project in 1977, because he was also an expert of population genetics. Some of the results (a karyographic analysis of mammalian karyotype evolution) were published in the American Naturalist as IMAI & CROZIER (1980) and IMAI & al. (1983). Later our theoretical works were compiled as the so-called "Minimum Interaction Theory" (MI-theory), which was submitted to the American Naturalist on 1 May (1985) and published as IMAI & al. (1986).

1985 was also memorable because a second initiative in ant cytogenetics started in that year. First of all, Taylor and I had been planning a large-scale chromosomal survey of Australian ants working out of Canberra (September to December 1985), with the support of CSIRO. While I was busy preparing for the journey to Australia, I received on 10 July a staggering manuscript from Michael W.J. Crosland, a Ph.D. student supervised by Ross. He had found a variety of "*Myrmecia pilosula*" with n = 1 at Tidbinbilla near Canberra on 24 February 1985. This was published in Science as CROSLAND & CROZIER (1986). Crosland informed me also that Graeme Browning who was a Ph.D. student at the University of Adelaide had been studying *M. pilosula* karyotypes independently.

Myrmecia pilosula, known as the "Jack jumper", had long been thought to be a single species. Ross and I examined its chromosomes in 1975, and found n = 5 or 15 / 16. Taylor and I extended utmost effort to find colonies of *M. pilosula* n = 1, but the *M. pilosula* we collected in 1985 had n = 12 ~ 16 as before. Later, Crosland sent me all his chromosome preparations and live cultures of the original material (Fig. 4). After much effort over several months on this material, I concluded that the *M. pilosula* with higher chromosome numbers was characterized basically by a complicated fission-fusion polymorphism (CROSLAND & al. 1988, IMAI & al. 1988). We were by now convinced that the so-called *M. pilosula* was not merely a single species but a species complex, study of which might provide the best supporting evidence for our MI-theory.

We carried out four expeditions gathering specimens and karyotypes of species assigned to the *Myrmecia pilosula* species complex (1987 ~ 1994) supported by a grant of the Japanese Ministry of Education. The n = 1 species was rediscovered at Corang river bridge (NSW) in 1987. To our surprise, the nest entrance was a tiny hole (ca. 1 cm) opening on bare ground and without a pebbly mound as was typical of other species. In the light of this knowledge a total of 19 colonies were located. They were found to have a complicated chromosome polymorphism (2n = 2 ~ 4 including 8 different karyotypes) and the 2n = 2 karyotype was seen to be induced secondarily from 2n = 4 by a telomere fusion (IMAI & TAYLOR 1989). They were clearly both karyotypically and morphologically different from other "*pilosula*". They were finally described as *Myrmecia croslandi* by TAYLOR (1991).

Taylor and I collected several hundreds of *Myrmecia pilosula* colonies based on a distribution map of *M. pilosula* prepared by BROWNING for his Ph.D. thesis (1987), which showed the species distributed broadly in New South Wales, Victoria, Tasmania, South Australia, and Western Australia. Meanwhile, Ross moved to LaTrobe University (Melbourne) as Professor of Genetics in 1990. He was kept too busy to join our field trips. I made efforts, however, to inform him about our progress. Retrospectively, Ross was the really indispensable interface between Taylor and me, because Taylor was unfamiliar with cytogenetics, as was I with taxonomic practices. These relationships were very important when we started to prepare a manuscript on *M. pilosula*-group chromosomes.

According to the karyological data, *Myrmecia pilosula* was seen to include at least five chromosomally distinct species, which were provisionally named by Taylor as *M. croslandi* (2n = 2 ~ 4), *M. imaii* (2n = 6 ~ 8), *M. banksi* (2n = 9 ~ 10), *M. haskinsorum* (2n = 12 ~ 24), and *M. pilosula* (2n = 18 ~ 32). A problem was that in my view, *M. pilosula* populations included several entities, *M. pilosula* s.str. and several putative hybrids with characters suggesting a gene introgression from *M. banksi*. I found an F1 hybrid karyotype between *M. banksi* and *M. pilosula* (PBF1, 2n = 5B + 14P = 19, where B = *M. banksi* genome and P = *M. pilosula* genome). Taylor and I now believe this entity to be a "good" species of hybrid origin, the description of which will shortly be published by Taylor. I also proposed that an intermediate color pattern of the legs found in the other putative hybrid populations provided morphological evidence suggesting gene introgression between *M. banksi* and *M. pilosula*. Taylor's comment was that "Frankly I am not sold on your hybrid hypothesis, and suspect that we in fact have a number of sibling species involved" (18 November 1993). As progress stagnated, I sent an e-mail to Ross (15 December 1993) that "This is an emergency! … I have previously told you that this paper should be published under the names of Imai and Taylor, and pointed out that you did not contribute to the central part of this project, however, if you check the ms carefully, you can. This would be good for you, because historically you initiated the *Myrmecia* project with Crosland."

Fig. 5: Ross H. Crozier (left), R.W. Taylor (centre) and H.T. Imai (right) at the IUSSI Adelaide Congress (Australia) in 1998 / 1999. Photographed by Y. Ugawa.

We then published the paper in the Japanese Journal of Genetics as IMAI, TAYLOR & CROZIER (1994). This was the last major paper of classical ant cytogenetics, because modern molecular genetics has dominated the field, overwhelming the classical methodology. We encouraged younger colleagues to investigate the *Myrmecia pilosula* species complex at the molecular level by using 28S rDNA (HIRAI & al. 1994, 1996), telomeres (MEYNE & al. 1995) and mitochondrial DNA (mtDNA) (CROZIER & al. 1995). All these molecular data, but especially the mtDNA data by Ross, supported the interspecies hybridization hypothesis. (The putative hybrids based on leg coloration are now believed by Taylor to represent a separate "good" species close to *M. pilosula* s.str.)

Our *Myrmecia pilosula* studies were reviewed in a poster session at the XIII International Congress of the International Union for the Study of Social Insects (IUSSI) (Adelaide, Australia) in 1998 / 1999, when Ross was President of the Union. This to my sorrow was the last time we met (Fig. 5).

Epilogue

After the IUSSI Congress, I started a new theoretical project (karyographic analyses of eukaryotic karyotype evolution by computer simulation) with population geneticists

Drs. Yoko Satta and Naoyuki Takahata (the Graduate University for Advanced Studies, Hayama, Japan). I asked Ross to join us again, because this work expanded the karyograph method we had published in 1980. He replied "I will make time to help with the ms and will not lightly withdraw my name from it. I am truly and deeply touched by your remarks, and will try to make this paper worthy of its place in your career" (25 August 1999).

Shortly after (2000), Ross moved to James Cook University (Queensland) as Professor of Evolutionary Genetics. He was exceptionally busy there, and finally wrote that "I have spent several days on the ms, but regret that I am not moving fast enough on it. I have a pileup of other mss and tasks, and I am not able to continue with this task, so must reluctantly return it to you" (18 March 2000). I understood that I had to achieve this work on my own, and completed it in 2001 (IMAI & al. 2001). Long afterward, I was told that Ross had said sorrowfully when he left the project that "I lost my old friend!" You misunderstood Ross, our friendship was much more durable than that!

I retired from NIG in 2003. Since then, I have been preparing a monograph of the MI-theory, which is approaching completion. I will dedicate this work to Ross.

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