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HISTORICAL & PERSONAL PERSPECTIVES

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# The discovery of the human chromosome number in Lund, 1955–1956

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**Abstract** The correct determination of the human diploid chromosome number as 46, by J-H Tjio and A Levan, at the University of Lund, Sweden, occurred 50 years ago, in December 1955; the finding was published in April 1956, ending a period of more than 30 years when the number had been thought to be 48. The background to the discovery and the surrounding factors are reassessed, as are the reasons why previous investigators persistently misidentified the precise number. The necessity for multiple technological advances, the power of previously accepted conclusions in influencing the interpretation of later results, and the importance of other work already undertaken in Lund, are all relevant factors for the occurrence of this discovery, the foundation for modern human cytogenetics, at this particular time and place.

## Introduction

The finding that the normal human diploid chromosome number was 46, rather than 48 as had been assumed for many years previously, represents the starting point of modern human cytogenetics, with great importance for future clinical applications in the detection of both constitutional chromosome abnormalities and somatic abnormalities such as those seen in cancers.

This discovery, made 50 years ago at the Institute of Genetics of the University of Lund, Sweden, in December 1955 and published early in 1956 in the journal *Hereditas* (Tjio and Levan 1956), is now of historical as well as of scientific importance. It is thus essential to have the details and circumstances of the discovery documented, and as far as possible agreed on, while some of those involved are still living. This is especially important as elements of controversy arose that made the authors of the publication, Joe-Hin Tjio and Albert Levan, both now deceased, reluctant to speak or to write about the topic in detail.

The present account draws on discussions with scientific workers at Lund during September 2004, as well as with others in the international cytogenetics community; also on articles previously written on the topic. Since the objective has been to document the scientific aspects of the study, not any personal factors, no attempt was made to contact any living relatives of those involved.

## The authors of the 1956 paper

Joe-Hin Tjio (Fig. 1) was born in Indonesia, of Chinese descent, and trained as a plant breeder (Hultén 2003; McManns 1997). The Japanese invasion of World War II disrupted his career; he was imprisoned and tortured both during the war and also afterwards as a communist by the Indonesian authorities. Colleagues speak of his bravery in helping others imprisoned. Tjio's links with Lund began as early as 1946 when, after moving initially to Holland, he came to the plant breeding Institute at Svalöf, near Lund, where he was involved in plant

**Fig. 1** Joe-Hin Tjio (1919–2001). From Hsu (1979), reproduced courtesy of Springer

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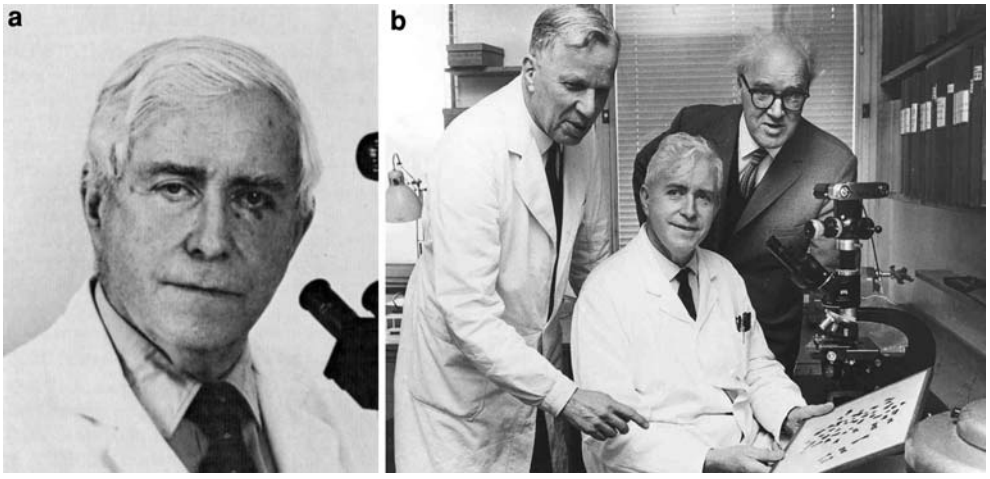


**Fig. 2** Albert Levan (1905–1998). **a** From Hsu (1979) courtesy of Springer. **b** With fellow Professors of Genetics in Lund, Arne Műnzing (*left*) and Åke Gustavsson (*right*) (Courtesy of Reinards Hochberg, University of Lund)

cytogenetic studies. The appointment of another Svalöf worker from Spain as head of an institute in Zaragoza resulted in an appointment for Tjio there, allowing continued working visits to Sweden. He and Albert Levan, also then based at Svalöf, were thus close colleagues for the 10 years prior to the 1956 discovery.

Tjio's later career, from late 1956, was in America. He moved with some reluctance, owing to the oppressive political climate of the time in the United States, but held appointments first with Theodore Puck at Denver, then at the National Institutes for Health, Washington. He died in 2001.

All those spoken with agree that Joe-Hin Tjio was a complex person, deeply affected by the traumatic experiences of his early life. He was a warm and loyal friend, but quick to see apparent slights or insults. Friends and colleagues describe recurrent episodes in later years, including difficulties with authorities arising from trivial incidents (Professor David Harnden 2004, personal communication). Throughout his life he was an avid photographer, not just on scientific topics.

Albert Levan (Fig. 2) was one of the key figures internationally in cancer cytogenetics (Hultén and Fredga 2003), founding a laboratory and tradition of cancer cytogenetics that continues to flourish today. A valuable video-recorded interview has been made in Lund in 1989 of his life and work, which gives insight into him as a person as well as documenting his scientific career.[1]

Levan's initial training was principally in botany; working at the Svalöf Plant Breeding Institute, close to Lund in southern Sweden, during the 1930s he made extensive studies of plant chromosomes, developing techniques of assessing chromosome damage from toxic agents on the root tip of dividing chromosomes of *Allium*, including the use of colchicine (Levan 1938). Impressed by the similarity of these changes to those in cancer cells, he changed his field to cancer cytogenetic research, moving to the Institute of Genetics, with a specific cancer chromosome laboratory established later, in 1953. He had extensive collaborations with US workers and in Lund trained many of the present generation of leaders in the field. His colleagues and others agree in describing him as a shy but warm person, held in great affection as well as respect. He continued working on cancer cytogenetics into old age, describing in the film that, after 50 years of looking at chromosomes every day, he regarded them as friends.

## Background and context of the research

Study of normal human chromosomes was not an active research field in the mid-1950s since it was generally accepted from studies over the previous 30 years that the number was 48 (Kottler 1974), while detailed study was limited by technology, as described below. However, Levan's extensive studies on the chromosomes of human cancers made it both important and necessary to be certain of the normal human karyotype. It is clear from both the film and Levan's overall work that a reliable and consistent determination of the human chromosome number was part of his wider research on cancer chromosomes. At that time there was no possible diagnostic use of chromosome studies in malformations or other human inherited disorders, nor was the Lund department set up to undertake such studies.

The same is also true for Tjio's previous work, which had largely been in plant cytogenetics. Thus, neither author was approaching the topic from the viewpoint of future clinical relevance outside the cancer field, something that may be overlooked today.

[1]This 1989 filmed interview, in Swedish, is with Bengt O.Bengtsson, now Professor of Genetics in Lund University. It is hoped that a version with English subtitles will be made.

## The technological context

That the discovery was made in the Lund laboratory is no chance occurrence, since it required the combination of a number of important technological factors; lack of any single one would have prevented or considerably delayed the successful experiments. A valuable historical article by Kottler (1974) has analysed these factors and their general significance in the interpretation of scientific results. Among the main factors are:

1. Use of the hypotonic chromosome spreading technique found (accidentally) by T.C. Hsu (Hsu and Pomerat 1953) and by others, and already in use in Lund by 1954. This was especially important in allowing unambiguous counts to be made of metaphase spreads.
2. Use of colchicine to arrest mitosis; a technique pioneered by Levan over 10 years previously (Levan 1938).
3. Use of cultured human embryonic cells; in the case of Lund, these were supplied by Professor Rune Grubb, head of the neighbouring university microbiology department. It is also relevant that Tjio and Levan were able to use these cells (cultured lung fibroblasts), with rapid growth, owing to Sweden being one of the few countries at that time where abortion was legal in certain circumstances.
4. Use of the 'squash' technique to bring chromosomes into a two-dimensional plane.
5. Use of photomicrography, allowing unambiguous counts on multiple cells and comparatively free from the inevitable biases resulting from the drawing of chromosomes. Levan was a strong advocate of the need for camera lucida drawings, while Tjio had considerable photographic skills. Both drawings and photomicrographs appeared in the published paper.

It can readily be seen that this combination of technological features, placed in the context of a well established and equipped cytogenetics laboratory, and involving experienced and skilled chromosome workers, was available in few, if any other laboratories outside Lund.

## Studies and factors leading up to the discovery

Although most of the scientific world was astonished to learn that the normal human chromosome number was 46, not 48, the findings in Lund did not occur out of nowhere. During 1954, Drs Eva and Yngve Melander, also working in the Lund Institute of Genetics, had studied chromosomes from embryonic liver tissue, using direct squash preparations, not cultured cells. As noted in the Tjio and Levan (1956) paper and subsequently by Kottler (1974) and by Hsu (1979), they found 46 chromosomes in their preparations.

Discussion with Eva and Yngve Melander in September 2004 confirmed this and they kindly showed me a photomicrograph of one of these preparations (Fig. 3), showing chromosomes well spread and a 46 count. They stated that they shared this information with Levan (but not with others) at the beginning of 1955 (not 1956 as implied in the Hereditas paper) and that Levan was convinced of these findings before he left for America in the Spring of 1955. It is possible that Levan may have tried to confirm the observations himself while in America, at Sloane Kettering Institute, New York, though in his report on this work, submitted to the journal *Cancer Research* in December 1955 (Levan 1956), he refers to the expected number as being 48. Why the Melanders' work was not continued is not clear, but it seems to have been incidental to their own principal research, and in discussion Eva Melander emphasised the superior quality of Tjio's preparations to her own. The findings were not published.

One point of relevance, in view of the extensive travels during 1955 of both Tjio and Levan, and in view of subsequent debate over respective roles, concerns the periods of time when they were present in (and away from) the Institute. It seems that Levan was away in America between May and August 1955, as noted in the book of Hsu (1979), but back after this until the end of the year. Tjio was in Lund between June and November

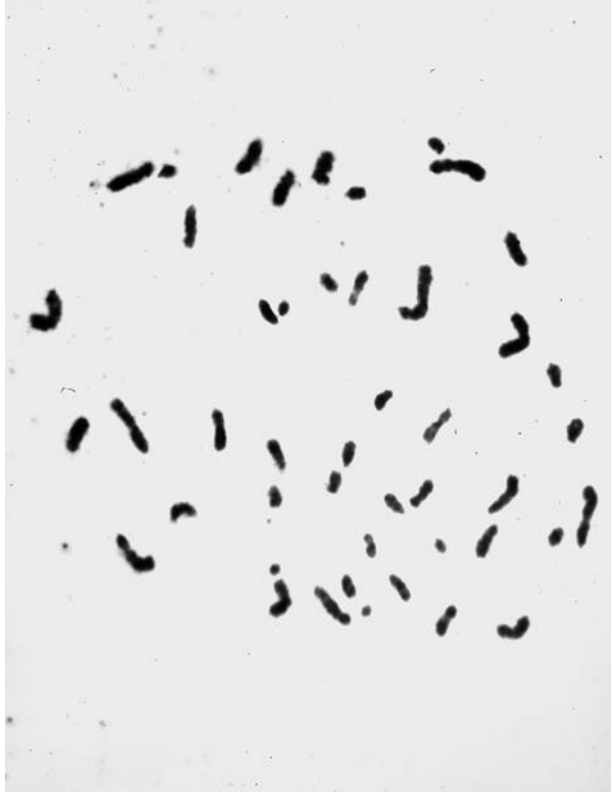

**Fig. 3** Human chromosome preparation by Dr Eva Melander, dated May 1954 and showing a 46XY karyotype. Previously unpublished and reproduced by kind permission of Dr Eva Melander, Lund

1955, and again between mid December 1955 and late January 1956. This indicates that Levan was present in the Institute of Genetics over the critical period of the discovery; Tjio's return to Lund only a short time before the date of the critical observation on December 22nd indicates that the final phase of the work was brief. This is not too surprising given Tjio's well-documented habit of working throughout the night, while the preparation of the cultured cells would have already been done in Rune Grubb's department.

A vivid account of the intense and intimate atmosphere in the Lund Genetics Institute at the time has been given by Professor Maj Hultén (2002), who spent time there as a project student from Stockholm over this critical period. This gives a valuable general picture, confirmed by others, showing how the Institute (Fig. 4) functioned as a busy teaching department by day, with most research beginning in the evenings and often continuing far into the night. It is clear that the small size and crowded nature of the cancer chromosome laboratory must indicate that any lack of communication between Tjio and Levan could not be attributed to physical barriers or distance.

## December 1955. The discovery

Photographic copies of the 46 chromosome number were later sent by Joe-Hin Tjio to friends and colleagues around the world, (Fig. 5) with a small label in the bottom left-hand corner stating in a variety of languages, 'Human cell with 46 chromosomes observed 1955 on December 22nd at 2.00 am'. This unusually precise documentation is important and also consistent, given the intensive and largely nocturnal work habits of Tjio and the various periods, mentioned above, when he was in Lund.

It is quite clear that the cytogenetic preparations and microscopic observations at this point were those of Tjio alone, distinct from any previous studies that might have been done. It also appears from a brief note written later by Tjio (1978), that Levan was not shown the results until after Christmas, something that suggests difficult relations between the two before there was any discussion over authorship, given the exciting nature of the results. Two colleagues, Carl Larsson and Maj Hultén, were, however, shown the preparations immediately (Hultén 2002).

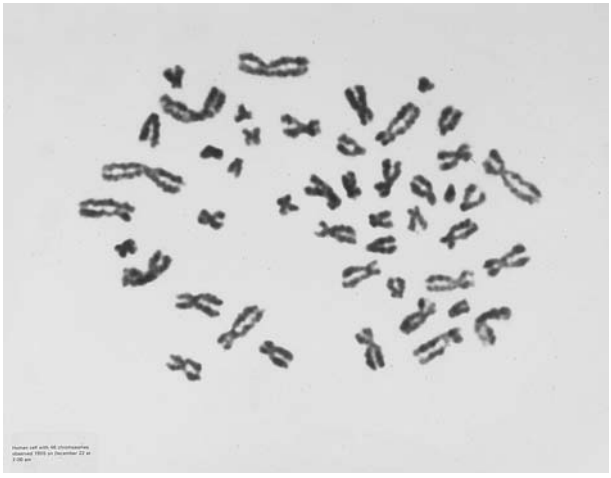

**Fig. 5** Photomicrograph to show the normal human 46XY chromosome number, as sent to colleagues by Joe-Hin Tjio (Courtesy of Professor Patricia Jacobs)

The high quality of the preparations as seen in the photomicrographs of the 1956 paper, the large number of cells counted (265), and the fact that only four cells showed a number other than 46, removed immediately any question of doubt as to the correctness of the findings. This conclusiveness of the results was of great importance given that they contradicted the number 48 previously accepted for over 30 years (Kottler 1974; Hsu 1979). As discussed later, workers who had previously supported the 48 number now found that their original material was actually consistent with 46 chromosomes.

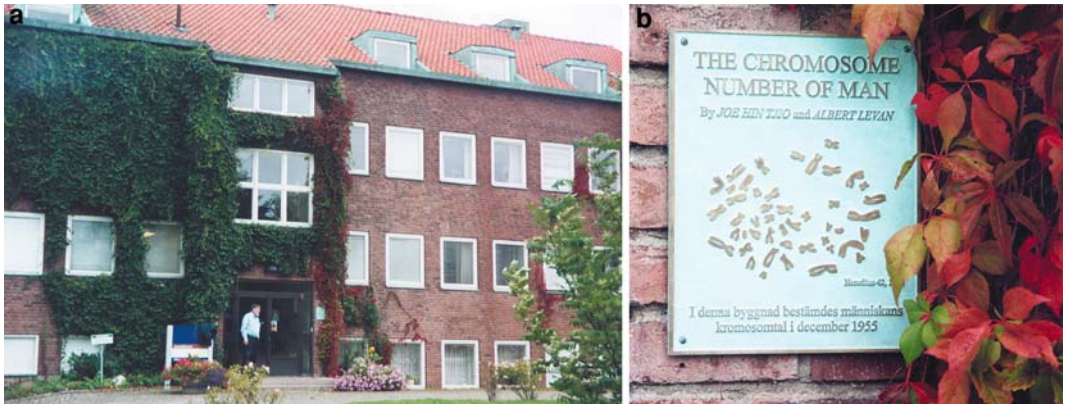


**Fig. 4** **a** The Institute of Genetics, University of Lund; photograph taken October, 2004, but the building is externally unchanged since 1956. **b** Plaque at the entrance to the Institute commemorating the determination of the human chromosome number (Photographs courtesy of Professor Ulf Kristoffersson)

## January 1956. The paper

The paper reporting these results, with Tjio and Levan (1956) as authors, was submitted to the journal *Hereditas* on 26th January 1956 and appeared in the April issue. Since *Hereditas* was published for the Mendelian Society of Lund and functioned as 'house journal' for the Lund Genetics Institute, it would not have received external refereeing, once approved by the head of the Institute, Arne Műntzing.

A significant disagreement seems to have occurred as to who should be first author of the paper. It has been suggested that Levan considered that he himself should be first author as being head of the unit, but in fact this does not seem to have been a universal or even widespread practice in Lund at this time. Frequently, a department or section head would not even be an author on a paper not directly involving them; Lund workers who had been later students of Levan cited examples of this from their own work, and acknowledgement in these circumstances was considered sufficient.

A more likely reason for the problem may have been that the work formed part of an important and long-running research programme of Levan, with his laboratory providing all the facilities; since he had initiated the project, obtained financial support for it, and had been involved conceptually throughout, he may well have felt that this merited first authorship.

The issue was rapidly resolved, but remained for Tjio a source of bitterness and later estrangement from Levan, these feelings persisting throughout his life as indicated in an interview not long before his death (McManns 1997). By contrast, the reaction of Levan seems to have more been, according to his Lund colleagues, one of puzzlement and sadness that such a dispute had arisen; the topic was little discussed in later years by him and the filmed interview gives no hint of tension or upset when the topic is raised. He makes it clear that his interests, once the correct normal number had been established, had returned to the primary topic of cancer cytogenetics.

As to who actually wrote the paper there is no direct information, but present members of the Lund Genetics Institute agree that it is written in 'typical Levan style'. Certainly, it reads as a paper written by a person experienced in writing scientific articles in English, clear in presentation and cautious in its claims, notably in its concluding sentence:

> ...we do not wish to generalise our present findings into a statement that the chromosome number of man is 2n = 46, but it is hard to avoid the conclusion that this would be the most natural explanation of our observations.

Given Tjio's preference for photomicrographs and Levan's for drawing chromosomes, it would be interesting to know whether the idiograms shown as Fig. 2 in the paper represent Levan's own observations and drawings. [For those without access to the original paper in *Hereditas*, it has been reproduced (with permission) in Harper (2004).]

It has been questioned why Professor Rune Grubb was not a co-author on the paper, given the essential role the cultured cell lines for the cytogenetic studies. Lund colleagues who interacted closely with him in later years state that he never referred to this topic; it would seem likely he would have considered the full acknowledgement for providing the resource given in the paper to be sufficient, since he was not himself involved in the chromosome analyses.

## Consequences of the paper

The clarity and unambiguous nature of the published results in the *Hereditas* paper meant that the predominant reaction internationally was surprise rather than dispute; confirmation rapidly came from other workers, (notably Ford and Hamerton (1956) at Harwell, England, using meiotic preparations from testis), while workers who had previously supported the number of 48, such as Hsu (1952) soon admitted that they had been wrong (Hsu 1979). Additional publicity was given in the form of a display by Tjio at the 1956 International Human Genetics Congress in Copenhagen (Fig. 6).

Debate rapidly turned to the question of how it was that repeated studies done over the previous 30 years had found 48, not 46 chromosomes. This is an important general issue for science, since it shows how, with the uncertainty resulting from inadequate technology prior to the 1956 study, a remarkable degree of subjectivity can enter into apparently unbiased analysis, later studies attempting to agree with previously accepted conclusions even when the facts did not justify this. Kottler's (1974) valuable historical study has analysed this in detail and the lessons to be drawn from it remain as relevant today as when it was written in 1974. A recent paper by Martin (2004) has examined this in the more general context of systems of counting.

At a more personal level, it has been mentioned that Joe-Hin Tjio moved in 1958 to Denver, where his colleague Theodore Puck convened the important 1960 conference establishing human cytogenetic nomenclature (Denver Report 1960). Invitations were confined to those who had published a normal human karyotype and it is interesting that 4 years after the original discovery, only three other European centres were represented (Edinburgh, Harwell, and Uppsala), along with three American groups and one from Japan, indicating that reproducing the technological and scientific skills present in Lund in 1955 was proving far from easy.

The Lund laboratory returned, as has been mentioned, to the study of chromosomes in cancer, but across the world the most profound and long-lasting consequence of the 1956 paper was to lay the foundations

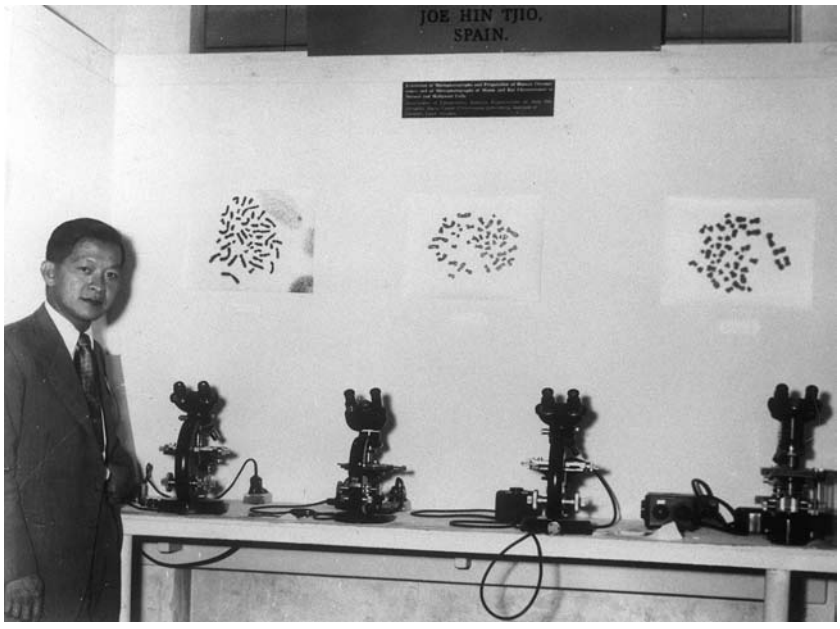


**Fig. 6** Joe-Hin Tjio demonstrating human chromosome preparations at the First International Human Genetics Congress, Copenhagen, August 1956 (Courtesy of Professor David Harnden)

for analysing constitutional chromosome disorders. Again it was a significant time (3 years) before these studies yielded results in the form of trisomy 21 (Lejeune et al. 1959) and sex chromosome anomalies (Jacobs and Strong 1959; Ford et al. 1959); not only had the technical factors to be reproduced in a medical setting, but methods of using less invasive samples, such as skin fibroblasts and peripheral blood, had to be devised before cytogenetics could become an integral part of the diagnostic analysis of genetic disorders.

## Conclusion

After 50 years, the paper of Tjio and Levan can be clearly seen as one of the major landmarks of human genetics, opening up the field of human chromosomes and of medical genetics generally to detailed analysis, as well as fulfilling its original aim of providing a normal reference point for studies of chromosomes in cancer. While personal differences were not absent from the discovery, the written and oral record of this important period is largely consistent and without significant disagreement. It reinforces the account shown by the published paper and confirms the necessary, though very different involvement of both Tjio and Levan as workers and authors, and of the underpinning foundations provided by the Lund laboratory, its other workers and collaborating departments. The role of those undertaking similar work in preceding years, notably the Melanders, and of other departments in developing essential techniques deserves also to be recognised, as we mark the 50th anniversary of this outstanding achievement.

**Acknowledgements** This paper has its origins in a series of recorded interviews with early workers in human cytogenetics during 2003–2004, and from an invitation by the University and the Mendelian Society of Lund to give their 2004 Nilsson-Ehle lecture. I should like especially to thank all those workers in Lund for their kindness, hospitality and for generously sharing their information and memories, concerning this important period. However, any inaccuracies or misinterpretations are entirely my own responsibility. Special thanks are due to the following Lund workers: Professors Bengt O. Bengtsson, Arne Hagberg (former director of Svalöf Institute), Ulf Kristoffersson, Felix Mitelman, and Drs Eva and Yngve Melander. Outside Sweden I am particularly grateful to Professors David Harnden and Maj Hultén for sharing their memories of this time.

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