Maarten Koornneef

ANNUAL REVIEWS

Annual Review of Plant Biology

A Central Role for Genetics in Plant Biology

Maarten Koornneef[1,2]

[1]Department of Plant Breeding and Genetics, Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany; email: koornneef@mpipz.mpg.de

[2]Laboratory of Genetics, Wageningen University and Research, 6708 PB Wageningen, The Netherlands

Annu. Rev. Plant Biol. 2021. 72:1–16

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

https://doi.org/10.1146/annurev-arplant-071720-111039

Keywords

Arabidopsis, natural variation, plant hormones, photoreceptors, genetics, autobiography

Abstract

This article describes my involvement in the development of genetics as an essential tool in the integrated study of plant biology. My research comes from a strong background in plant genetics based on my education as a plant breeder at Wageningen University and collaborations with plant physiologists and molecular geneticists in Wageningen and the wider scientific community. It initially involved the isolation and physiological characterization of mutants defective in biosynthesis or mode of action of plant hormones, photoreceptors and traits such as flowering time in both *Arabidopsis* and tomato. I also generated a genetic map of *Arabidopsis*. Subsequently, the exploitation of natural variation became a main area of interest, including the molecular identification of underlying genetic differences. The integration of various disciplines and the adoption of *Arabidopsis* as a main model species contributed strongly to the impressive progress in our knowledge of plant biology over the past 40 years.

Contents

EARLY YEARS AND EDUCATION

I was born in 1950 as the oldest son in a family with seven children in De Lier, a village in the west of the Netherlands. This region, south of The Hague, is called the Westland. It was and remains a center of horticulture, covered with greenhouses. Initially, my father grew vegetables on a small allotment that he rented. Ten years later, we moved to the neighboring village of Maasland where he bought his own nursery, expanding this over the following years. Both of my parents came from large families in which there was no tradition or possibility of having a higher education. Neither they nor any of my uncles and aunts attended secondary school, and my parents did not speak English. However, by hard work and the adoption and development of innovations, as well as having green fingers, they were able to earn a good income. This meant that their children's educations were not limited by a lack of finance, and a scholarship was unnecessary when I went to university. Nevertheless, at the time, higher education and university were not expected of me. After elementary school, I did not go to a secondary school that prepared students for university, but to one (called a middle school) that equipped students for a more practical career. In fact, only 1 pupil out of 30 in my primary school class went to the university-preparatory secondary school. However, the good thing in the Dutch education system was (and is) that able pupils can move to higher school systems after completing their education at the lower level. This leads to the loss of a year or even more, but in exceptional cases it can lead to careers, even in science, without a regular university education. After my three years in the middle school, I went for a further three years to the high school that gave me access to Dutch universities. I had little spare time because as a teenager I worked in my father's nursery during most of the school holidays, as well as before and after school (**Figure 1**). However, some time was available for sports and for borrowing books from the public library. Plants were, to us, the way to earn a living, and I certainly knew about how plants grew and developed. However, I did not associate this with plant biology, as such, or natural history. Plants were certainly not my hobby, and I did not show a great interest in nature at that time. The books I read were about history and geography. At primary school, I made atlases and wall maps by enlarging parts of smaller maps. I had no idea that different types of maps would play such a prominent role in my future career.

After I completed secondary school, I decided not to study history or geography, mainly because I thought my only option would be to become a secondary school teacher. I also liked biology, and because I was familiar with horticulture, I chose to study agriculture at the Landbouwhogeschool in Wageningen (now Wageningen University and Research), the only Agricultural University in the Netherlands. This was my own choice, but it was completely supported by my parents. Being a student in the small town of Wageningen (30,000 inhabitants) was very enjoyable. Most of the

Figure 1

Harvesting tomatoes in my father's greenhouse as a teenager.

time, I shared a student apartment (individual rooms for eight students with a common kitchen and bathroom), and my social life was with my neighbors and within student unions, as well as with colleagues studying similar topics.

After a first year with everyone studying the same curriculum, we had to choose a specific area of study. Although initially I thought about horticulture, I chose plant breeding. This topic was, according to fellow students, more focused and also intellectually more of a challenge. Students obtained a solid education (both theoretical and practical) in many aspects of plant breeding and genetics, as well as in plant pathology and statistics. In retrospect, I missed out on biochemistry and molecular biology. The latter was treated only as a subtopic of genetics. A Master of Science degree (MSc) consisted of research projects and literature reviews. I did this for plant breeding (my major), studying hybrids of *Streptocarpus* species, and I did minors in horticulture, plant taxonomy (mainly, in my case, analyzing chromosomes of *Alstroemeria* species), and genetics. During my 3 months in the Genetics Department, I worked, for the first time, with *Arabidopsis* under the supervision of Professor Jaap van der Veen, my future PhD supervisor. The project dealt with testing mutagenic treatments and analyzing them using Müller's embryo test. One of the MSc requirements was a 6-month internship to experience plant breeding in practice. I spent 3 months at a vegetable-breeding company in The Netherlands and 3 months in the UK at a company that bred and multiplied special ornamental plants. Nowadays, such internships are generally done in research institutes abroad. After 6 years, I obtained the title Agricultural Engineer (ir), comparable with an MSc. This was a successful time of study for me, and both my BSc and MSc diplomas were awarded cum laude.

In Wageningen, I met Elly Scheps, a food technology student, and we married in 1974 at the end of our studies (two days before my final exam in plant breeding). We have two children and four grandchildren. Our son Wietse studied building physics at Delft University, a field in which he still works. Our daughter Annemart studied biology at Utrecht University and did her PhD in

plant pathology on *Arabidopsis*. Her supervisors were Cees van Loon and my former MSc student Corné Pieterse. Thereafter, she started working for medical research companies.

AFTER UNIVERSITY

Although I enjoyed research, continuing my work with the goal of earning a PhD was not an obvious choice. In 1974, very few or no PhD positions were available in my specialities. Research positions that did not require a PhD were not available at that time, but breeding companies were keen to employ Wageningen plant-breeding students. A year before I graduated, I had already received three invitations to apply to different companies. Growing companies needed specialists in plant breeding to address complex projects, such as breeding for disease resistance and the development of hybrid varieties. I accepted a job as a plant breeder at Vandenberg seeds, a medium-sized company breeding and selling vegetable seeds. I was their first employee with a university degree and was accepted fully by the people working there. The head of plant breeding needed my knowledge and was very happy to share with me his know-how on the organization methods of breeding and what the perfect crop should look like. I started new programs on bell pepper and eggplant and intensified the ongoing breeding programs. When I left two years later, I had produced a successful eggplant hybrid variety, and many of the other breeding programs were well underway. The company immediately appointed my successor, a fellow student, who happened to be my second cousin. The reason that I left was a telephone call from Jaap van der Veen, the Professor of Genetics at Wageningen, telling me that there was a vacancy for a scientific staff member in his department. This was, in principle, a permanent position and would give me the opportunity to obtain a PhD. It meant that I became an assistant professor without having a PhD. Van der Veen invited me to apply for this position after consulting the Professor of Plant Breeding. They had decided that I was the best available candidate among the recently graduated plant-breeding students. Van der Veen was a botanical geneticist, as well as a population and quantitative geneticist. He trained in the latter topics during a postdoctoral position with Professor Mather in the United Kingdom after obtaining his PhD on the genetics of leaf shape in tobacco in Wageningen. When I arrived in 1976, his research dealt with projects that were suitable for short (3 months) MSc projects for the many students that chose genetics as a minor study. I took over these projects, except for those dealing with late-flowering mutants, which he used as a model for quantitative genetics. The most promising and novel project that I took over was the study of newly discovered gibberellin mutants in *Arabidopsis*. Stimulated by an article showing that plant hormones were important for all aspects of plant development, including seed germination, van der Veen hypothesized that mutants affecting plant hormone concentration action would have a germination defect and should be looked for among nongerminating mutants. They could then be rescued by applying the plant hormone. This idea was tested by Agnieszka Barbaro, a visiting scientist from Poland. In the summer of 1976, she had identified two such mutants in which a nongermination phenotype could be rescued by gibberellic acid (GA). This resulted in a dwarf plant that could be restored to a plant with the height of the wild type by spraying with GA. I immediately started further mutation induction and screening experiments using ethyl methanesulfonate to identify additional mutants. I could also screen for interesting mutant phenotypes in irradiated mutagenized populations generated by Lidwine Dellaert, who was the only other PhD student working with van der Veen on a comparison of mutagenesis induced by X-rays and fast neutrons. The neutron source was available in Wageningen but was about to be closed. One of the scientific justifications for the closure was that with the much more simple X-ray facilities, one could conduct the same mutagenesis experiments. It was expected that when I obtained publishable results, these could be used for my PhD. However, there was no urgency for this since I had a position with the university, which after a few years would become permanent.

In 1977, we published our work on the isolation of gibberellin-sensitive mutants in *Arabidopsis*, comparable to those already identified in maize and rice (although these have no germination phenotype). This short note in the *Arabidopsis* newsletter Arabidopsis *Information Service* (*AIS*) (21) was my first publication. From then onwards, I published such preliminary data in the annual issues of *AIS* until this newsletter closed down in 1990, when Albert Kranz retired. Some of these short notes were cited, e.g., the paper describing the *transparent testa glabra* (*ttg*) mutant and the seed mucilage phenotype of this and of some other mutants (20).

Although van der Veen was very interested in my research, which I did in addition to light teaching duties, he gave me a lot of freedom in deciding how to organize my own work. The many MSc students (31 in the first 6 years) who did short projects with me all took their exam with van der Veen and me. This provided him with updates of my research, as did our frequent discussions. The second student project that I took over was the study of trisomics in *Arabidopsis*. These could be used to map genes, based on their mutant phenotype, to chromosomes. The *Arabidopsis* linkage maps were limited, except for chromosome 2, which was published by George Rédei (see 19), who had also defined the linkage groups with one to three morphological markers each. To perform trisomic mapping, I was able to make use of the collection of morphological mutants that van der Veen had collected over many years, which I expanded with mutants I had isolated. These included the homeotic floral mutants that I later sent to Elliot Meyerowitz at the California Institute of Technology. After assigning genes to chromosomes, the obvious next step was to intercross mutants located on the same chromosome and to study their linkage. To use the recombination estimates with their standard deviations, I made use of a method that had just been published by Jensen & Jørgensen (14). This method required a maximum-likelihood analysis, which soon became so complex that it required computer support. Fortunately, Piet Stam, a colleague within the department, had the skills to do this. It stimulated him to develop methods for generating genetic maps and later, together with his coworkers, for quantitative trait locus (QTL) analysis. This resulted in the JoinMap program (43). When we tried to publish the first complete genetic maps with 76 genes, using mainly our data but also some from the literature, we had problems in getting the paper published in the *Journal of Heredity* because they found the large tables with a lot of recombination data unnecessary. This was before the era of supplementary online data. One striking remark by one reviewer was, "A much shorter article based on one table and figure (the map) would be far more acceptable." Somehow, *Arabidopsis* failed to excite as many genetic researchers as we had anticipated in the 1960s. I wrote a rebuttal letter saying that the linkage of a single segregating progeny in mice had been published in the same journal and that *Arabidopsis* was gaining interest, citing recent papers, for example, by the Somervilles. Finally, the journal decided to publish the paper as originally submitted, due to, as stated in the final acceptance letter, my persuasive letter (31). This felt like a personal victory for a young PhD student. I considered this mapping effort mainly as a teaching tool for students, and most of the emphasis was on the study of plant hormone mutants, which led to two important new lines in my research. I decided to mutagenize the gibberellin mutants and to select for mutants that germinated without GA application. This resulted in the identification of abscisic acid (ABA)-deficient mutants acting as suppressors of the nongerminating phenotype. I remember that the eye-opener for me was seeing a mild wilting phenotype, reminding me of the wilting ABA-deficient mutants in tomato described by Moshe Tal, in the F2 progeny of the cross revertant (double mutant) × wild type. This was not a strong phenotype, and the MSc student who worked on this could never recognize it. Having a good eye for plants helped me a lot, and instead of spending time in the laboratory and at the desk, I was often found in the greenhouse (**Figure 2**).

Sometimes we invited other botanists from our university to have a look at our mutant collections. On one occasion, I showed some mutants with long hypocotyls to Professor Joop Bruinsma,

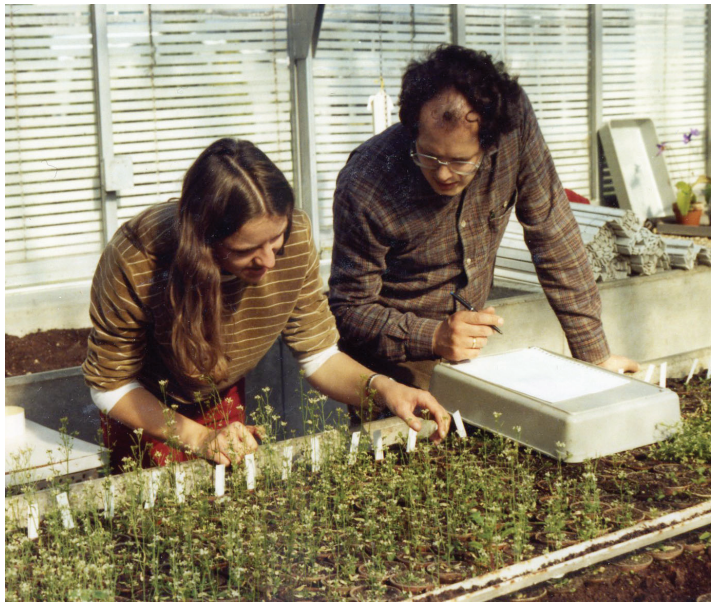

Figure 2

Scoring plants in the greenhouse with my technical assistant Corrie Hanhart in 1980.

Professor of Plant Physiology. I had an interest in these mutants, because they looked like the opposite of GA dwarfs and they could be overproducers of gibberellins. However, an eye-opener was Bruinsma's remark that they looked like dark-grown plants. I linked these plants immediately with facilities in the laboratory of Carl Spruit at another plant physiology department in our university. There I was investigating the light effects on the germination of GA mutants, testing the idea that light acts via GAs. I had seen that Spruit had cabinets with different light colors, and I started growing the long hypocotyl mutants in these cabinets. This gave very clear results, with some mutants insensitive to inhibition by specific colors. Spruit could measure phytochromes spectrophotometrically, and it appeared that some mutants contained no detectable amounts of phytochrome. We were excited but also worried that many specialists in the phytochrome field would not believe that plants can live without phytochrome. To avoid critical reviews, we sent our paper to a plant physiology journal with a lower impact, in which it was accepted without problems (28). When I came to the United States 5 years later, people were aware of this paper, but the rumor had spread that we had just measured chlorophyll instead of phytochrome. Initially, this paper was hardly cited, but later on it became one of my most-cited papers, with a maximum number of citations per year 15 years after the paper was published. It apparently was a bit too early for this paper, which perhaps should have been published in a journal with a much higher impact. An important finding was that we could suggest that *hy4* mutants might be blue light receptor mutants, which later on was shown by the cloning of the gene by Ahmad & Cashmore (3). The clear aim of these hormone and mutant studies was to understand plant physiology. I called myself in those days a physiological geneticist. A nice example was the work we did, together with Cees Karssen (17), a staff member at the Laboratory of Plant Physiology (16), on the role of ABA in seed germination. Karssen's laboratory could also measure ABA concentrations and did so for the various ABA-related mutants. It was obvious that we needed to understand the biochemical lesions in the mutants. This required specialists capable of measuring hormone levels. Fortunately,

Figure 3

Professor Jaap van der Veen and Dr. Cees Karssen during the defense of my PhD thesis in 1982.

I met Jan Zeevaart (48) in 1982, when he was visiting his sister-in-law, who lived in Wageningen. His laboratory in East Lansing, Michigan, United States, could measure both ABA and GA levels, and he used his expertise to biochemically characterize some of the GA and ABA mutants. This fruitful collaboration meant that I frequently visited the Plant Research Laboratory in East Lansing, where I met many other very good plant scientists. I often visited Jan on my way to the *Arabidopsis* meeting in Madison, and several times we drove there together, all the way from East Lansing. Initially, I was not aware of another application of these mutants: They could be used to clone the underlying genes by, for example, map-based cloning. In fact, the *ABI3* gene was one of the first genes that was map-based cloned by Jerome Giraudat (12) on the basis of the map position we had published in AIS (23). This was information that we had deleted from our paper (27) after the suggestion of Karssen, because he did not see why this mapping information was useful in a paper describing ABA-insensitive mutants. Another gene-cloning technique developed by Tai Ping Sun and Fred Ausubel was deletion cloning, which they demonstrated with one of our *ga1* mutants. We had previously shown by intragenic recombination studies that it might have a large deletion (30, 44). By 1982, I had published four papers, and another four papers were submitted or ready for submission. This was more than sufficient to bundle together as a PhD thesis, which I defended cum laude in November of that year (**Figure 3**).

MOVING TO PLANT CELL AND TISSUE CULTURE AND TOMATO

After obtaining my PhD degree, I realized that it was a good time to change the direction of my research. It was a tradition that staff members of the Genetics Department could go abroad for a sabbatical/postdoc. Plant cell and tissue culture research was booming at that time, particularly in applications for vegetative propagation of plants but also in genetic applications such as somatic hybridization and transformation, techniques which required protoplast isolation and culture. In 1980, at a conference in Vienna, I met Christiane Gebhardt, a PhD student of Patrick King from the Friedrich Miescher Institute in Basel, Switzerland, and Pal Maliga, who discussed their work on the isolation of mutants using protoplasts from haploid plants. This technology could give me

access to mutants with affected auxin and cytokinin biosynthesis, which I saw as following up on my work on GA and ABA mutants. I contacted Pat King, and in February 1983 my wife and I drove to Basel with our two young children (2 and 4 years old) for a fruitful 6-month stay. In addition to allowing me to learn tissue culture technology, the sabbatical in Basel showed me that science was international and that contacts were essential. At that institute, new developments were known, long before papers were published, because the staff attended many conferences and reported about them. In addition, important scientists visited and discussed ongoing research. Back in Wageningen, I started new projects, realizing that *Arabidopsis* was not ideal for tissue culture and producing haploids was impossible (according to failed attempts in other laboratories). Tomato seemed a better option, and, in addition, it was a good plant for genetics, as well as being an important crop species, especially in the Netherlands. Furthermore, the Genetics Department had relatively large greenhouse facilities and experience with this plant that could be useful for mutagenesis and genetic studies. I realized that tissue culture worked better in the wild relatives of tomato. In Wageningen, Dr. Hoogeboom had made hybrids between the cultivated tomato and one of the wild species with favorable tissue culture properties, so we started breeding for tissue culture response (24). We also identified a tomato haploid (29). The better regeneration capacity of the wild relatives of tomato provided the basis of my first grant proposal submitted to the Dutch Research Council (NWO). I planned to obtain asymmetric somatic hybrids by fusing irradiated protoplasts from the wild species with protoplasts of the cultivated tomato and selecting hybrids on the basis of their regeneration capacity. This successful project was begun with Jelle Wijbrandi, my first PhD student, who graduated in 1989, and was continued by Anne-Marie Wolters and Herman Schoenmakers, two new PhD students, funded by a biotechnology program in the Netherlands. In addition, I came in contact with Dr. Pim Zabel, at the neighboring Department of Molecular Biology, who had started a project on the cloning of the nematode resistance gene *Mi* in tomato. I provided the genetics know-how (e.g., 46) and attended their weekly laboratory meetings where I learned a lot about molecular genetics, including its technical problems. Together, we also developed transformation technology in tomato, where I was responsible for the tissue culture aspects (15, 24). Tomato had also become a topic for physiological genetics because we decided to see if similar hormone and photoreceptor mutants could also be found in tomato. These were easier to study than *Arabidopsis* because of their larger size. Phytochrome-deficient mutants were already available as *aurea* and some *yellow-green* mutants. We categorized them with help from Dick Kendrick, the successor of Carl Spruit and my long-standing collaborator on photoreceptor mutants (22), and also with the group of Peter Quail (37). Later on, we isolated additional mutants that were characterized by our PhD students and colleagues in Japan, where Dr. Kendrick was leading a Frontier Research project as part of the Riken organization, while also maintaining his position in Wageningen. Altogether, I was involved in many papers on tomato, especially in the 1980s and 1990s. I also enjoyed the interaction with tomato geneticists such as Charley Rick and Roger Chetelat from the University of California, Davis, and Dani Zamir at the Hebrew University of Jerusalem, as well as with the Wageningen groups.

BACK TO *ARABIDOPSIS* AND ADDING MOLECULAR GENETICS TO OUR TOOLBOX

After my PhD and sabbatical in Switzerland, I still had previous *Arabidopsis* research that had not been published. By 1991, I had published 10 *Arabidopsis* papers in refereed journals, together with at least one short article in *AIS* every year and a number of reviews, such as symposium proceedings. These were often in collaboration with others, including Cees Karssen, Jan Zeevaart, Dick Kendrick, and Gerard Barendse from Nijmegen University. In those days, the acceptance of

genetic research in plant physiology studies was still limited. The paper on ABA-insensitive mutants, which contained various physiological experiments and measurements of ABA content, was rejected by *Planta*, after 3 months and without review, because it was a genetics and not a plant physiology paper. The next attempt to get it published in *Theoretical and Applied Genetics* failed because it was on *Arabidopsis* and not a crop plant, despite the fact that earlier descriptions of GA- and ABA-deficient mutants were happily accepted by the same journal. However, the third attempt to get it published in *Physiologia Plantarum* was successful, and this paper later became my second most-cited paper (27). The remaining *Arabidopsis* experiments I conducted myself, together with my technician, because the PhD and MSc students were dealing with cell genetics and tomato experiments. Van der Veen retired in 1988 and handed over to me the *Arabidopsis* project on late-flowering mutants that he had kept for himself. Earlier, I had collected additional late-flowering mutants and included them in our mapping experiments. I performed some additional physiological experiments with these mutants and published a paper in *Molecular and General Genetics* with van der Veen and my technician Corrie Hanhart, which is my most-cited paper (25).

Arabidopsis research had been started by Friedrich Laibach in Germany with his review in 1943 (33). Wil Feenstra introduced this plant to the laboratory of Genetics in Wageningen in 1962, when he brought seeds with him from G. Rédei. The revival of *Arabidopsis* research started in the United States. This history has all been described in several reviews (26, 36, 39, 41). Our papers on *Arabidopsis* in the 1980s were published on the right topics at the right time, and I benefitted from this, as did, I hope, the *Arabidopsis* research community.

The reasons why I changed my research to tomato were partly because of some frustration. Although in my opinion I did interesting work on *Arabidopsis*, it did not attract much attention. The renaissance of *Arabidopsis* research only became clear to me when I was invited for the Keystone Symposium on Plant Genetics in 1985. In the period before that meeting, I had corresponded with various scientists from the United States. This gave me sufficient contacts to organize a trip of almost a month to various laboratories following the conference. The conference, and my first journey to the United States, was an impressive experience. It showed me how plant science was developing and how *Arabidopsis* would play a central role. At this meeting, I met the revival pioneers of *Arabidopsis* research, whom I had only known from their papers and correspondence, and it was nice to meet them personally (**Figure 4**).

The picture in **Figure 4**, taken by an unknown attendant with my camera, was published in the Meyerowitz historical review (36) and in a review by Sabina Leonelli (34). The latter suggested that this was the occasion where we organized *Arabidopsis* research, although I do not remember this. *Arabidopsis* research eventually became organized, and this was mainly achieved in committee meetings during a new series of annual conferences that started in 1987 in East Lansing. This resulted in a multinational *Arabidopsis* Steering Committee (MASC), which also laid the foundation of the *Arabidopsis* genome project, an initiative of the National Science Foundation (NSF) of the United States, which supported workshops in 1989. These projects were discussed during the MASC meeting at the *Arabidopsis* conference in Bloomington (40). In April, an ad hoc committee met in Denver and wrote a document describing the plans for a genome project from 1990 to 2000. This was further discussed during the *Arabidopsis* conference in Vienna, which included representatives of the European Union (EU). The impressive document "A long-range plan for the multinational *Arabidopsis thaliana* genome research project" was published in 1990 by the NSF, and it predicted that the whole-genome sequence would be ready by 2000. This was despite the fact that no details could be given for the goals in the final 5 years (1995 to 2000) because new technology was needed (which arrived in time). The East Lansing *Arabidopsis* conference in 1987 had 217 participants, and there was an open and optimistic atmosphere where people talked freely about their plans and showed their results. I found this open atmosphere very rewarding, and the

Figure 4

(*Left* to *right*) Shauna and Chris Somerville, Elliot Meyerowitz, David Meinke, Marta Crouch, and me at a break during the Keystone meeting in 1985.

annual *Arabidopsis* conferences in the 1990s, mainly in Madison, Wisconsin, were a pleasure to attend. They involved a lot of discussion and making of agreements on collaboration during the poster sessions and afterwards when having drinks on the Memorial Union Terrace.

In retrospect, it was in the 1980s that the basis for *Arabidopsis* research was put in place. This came to fruition in the 1990s, leading to the landmark paper on the genomic sequence published in 2000 (7). I was not involved in this sequencing project at all; however, we benefitted from this work, as did everyone working with *Arabidopsis*, when publications on gene isolation started appearing. From 1990 onwards, the number of papers on *Arabidopsis* significantly increased every year (41).

These international developments were also very important for my research, resulting in extra funding. This was especially true in Europe, as in 1991 the EU launched the BRIDGE (Biotechnology Research for Innovation, Development, and Growth in Europe) program, led by Mike Bevan (35). This project was initiated at a meeting in Brussels, where I had been invited as part of an ARABESK group (35). This is where I met the leaders of plant science in Europe, such as Dick Flavell, Jeff Schell, and Marc van Montagu, for the first time. One important question for me was which *Arabidopsis* genes are also important for crops. I mentioned the gene underlying the GA-insensitive mutant *gai*, which could be the same as the green revolution genes in wheat. This was later shown convincingly to be the case by the group of Nick Harberd, who first cloned *GAI* and, with this sequence in hand, the wheat homologs (38).

I was involved in two BRIDGE projects, which meant that I could appoint a postdoc, for the first time, who had expertise on molecular genetics. Anton Peeters started in my laboratory, working on the cloning of one of the genes underlying the late-flowering mutants that had been described in Wageningen (25). He took part in the floral induction project with José Miguel Martinez-Zapater, George Coupland, Caroline Dean, and van Montagu's group. I could also appoint Karen Léon-Kloosterziel as my first PhD student working on *Arabidopsis*, as part of the

project on ABA. The flowering time project received additional momentum when, in 1993, Wim Soppe started his PhD research on this topic, together with Carlos Alonso-Blanco, who came with a Spanish fellowship in the same year. This finally led to a successful cloning of one of the few epigenetic mutants in *Arabidopsis*. Due to Wim's perseverance, it was shown that the two *fwa* mutants did not contain a nucleotide change, but were, in contrast to the wild type, much less methylated in the promoter of the *FWA* gene (42).

Having extra people with their own funding was a great help, and a nice side effect was that it made the group very international. We also benefited from the additional *Arabidopsis* research in Wageningen. The BRIDGE program included the group of Andy Pereira working at one of the Wageningen plant breeding institutes of the ministry of Agriculture [this was part of an organization similar to the US Department of Agriculture and the National Institute for Agricultural Research (INRA) in France]. The group of Andy Pereira and Willem Stiekema became part of the BRIDGE program despite the fact that work with *Arabidopsis* should not have been conducted in their institute. However, there was extra money for it, which was used to employ Mark Aarts as a PhD student. His research resulted in the first *Nature* paper of that institute (2), on the cloning of a male sterility gene using transposon tagging. Because the institutes needed a university professor as a formal PhD supervisor, I was asked to be the so-called promotor for Mark. This had become possible because the leadership of the Genetics Department had successfully proposed that I should receive one of the personal professorships that became available at Wageningen University in 1992. Mark and Andy also provided us with one of their transposon-induced mutants, which was then used by Hiroshi Kubo, a visiting scientist from Japan. He cloned the underlying gene, which was the subject of the first cloning paper from our laboratory (32). Another postdoc, Isabelle Debeaujon, who came with a personal EU fellowship, started to work on seed color mutants (10), work she still continues in France. In 2001, Mark Aarts joined my group as an independent researcher focusing on tolerance of heavy metals in *Arabidopsis* relatives and various projects on the use of natural variation in *Arabidopsis*. Nowadays, this research also has an emphasis on photosynthetic variation. This input, from several highly talented postdocs and PhD students, made the period around 2000 very productive. This was especially true because it was when we initiated a new topic, which was natural variation in *Arabidopsis*.

NATURAL VARIATION IN *ARABIDOPSIS*

Although Prof Laibach had championed the use of natural variation in *Arabidopsis* (33), and Jaap van der Veen had also started some experiments with natural accessions for the study of flowering time variation, my early work on *Arabidopsis* only dealt with mutants in the Landsberg *erecta* (L*er*) genetic background. However, in the 1990s I received a sample of the *Arabidopsis* accession Cape Verde islands (Cvi) from George Coupland, which, according to George, was relatively insensitive to daylength. When I received the seeds, I noticed that they were much larger than Landsberg seeds, and because Karen Léon-Kloosterziel was looking at some seed size mutants, I decided to plant them and crossed Cvi with L*er*. When Carlos Alonso-Blanco decided to stay longer in Wageningen and to apply for an EU fellowship, I suggested that he should investigate the genetics of natural variation, using the Cvi/L*er* recombinant-inbred line (RIL) population that we were developing. This was the best advice I ever gave, and Carlos became a real pioneer on this topic. He described the methods for this in his review in 2000 (6). He initiated the research to work out the genetics of seed size differences and some related traits such as ovule number (5). This paper was used as an inaugural paper for *PNAS* when I was elected as a member of the National Academy of Sciences in 1998. We were lucky that Piet Stam and his coworkers Ritsert Jansen and Johan van Ooijen in Wageningen had developed not only computer programs

to make genetic maps but also methods for QTL analysis. To validate these programs with real data, I mediated a collaboration between the Stam/Jansen group and Caroline Dean to use their flowering time data in a Columbia (Col) × L*er* RIL population, which they had made before we started our natural variation project (13). We used the Multiple QTL Mapping (MQM) program developed by Ritsert to analyze seed germination data obtained with the same Col/L*er* population as part of an MSc project (45). Carlos was involved in writing the paper and quickly became familiar with the methods to analyze such data. When we were growing the Cvi/L*er* RILs, we also noticed the large variation in flowering time, as well as differences in seed dormancy. These were topics that could be used for PhD projects. Flowering time was worked on by Salah El-Din El-Assal, who came with a fellowship from Egypt and who cloned one of the first QTLs in higher plants (11). I obtained a PhD fellowship from the Dutch science foundation to work on seed dormancy and appointed Leónie Bentsink. She later obtained a postdoc EU fellowship in the international *Arabidopsis* natural variation project called Natural that I coordinated. As part of that project, Leónie cloned the *DOG1* QTL (9). This gene appeared to be very important in seed dormancy, and it was further studied by her and the group of Wim Soppe when he became group leader at the Max Planck Institute for Plant Breeding Research in Cologne. In their first year, both Salah and Leónie were supervised by Carlos. He left for Spain at the end of 1998, where he started a successful program on natural variation, with an emphasis on material that he collected in Spain. He also became part of the EU Natural program. One interesting side effect of working with natural variation is that one becomes an *Arabidopsis* collector (**Figure 5**). I collected many accessions on holidays in the Netherlands and abroad, but the champion of this was Carlos, who built up a very important collection from Iberia and Morocco. This became the basis of a lot of ecological genetics, as well as being important when the resequencing of *Arabidopsis* accessions was done (1). Another very active collector was Padraic Flood, an Irish PhD student in Wageningen, who collected extensively in Ireland and Africa (as a member of the group of Angela Hancock). Research in Wageningen also developed a close collaboration with Dr Dick Vreugdenhil, a plant physiologist interested in metabolic variation. In Wageningen, Joost Keurentjes, who was one of my last PhD students, was cosupervised by Dick and worked

Figure 5

Collecting *Arabidopsis*: me in the Netherlands (*left*), Carlos Alonso-Blanco in Spain (*middle*), and Padraic Flood in Ireland (*right*). Middle photograph provided by Carlos Alonso-Blanco. Right photograph provided by Padraic Flood.

on omics variation in the Cvi/L*er* population (18). Joost, together with Erik Wijnker, another Wageningen PhD student, organized a competition on collecting *Arabidopsis*, both within the genetics laboratory and via a nature program on the national radio in the Netherlands. All these materials were the basis of further genetic and ecological evolution studies, especially powerful when using genome-wide association mapping of fully sequenced genomes.

FINAL YEARS IN WAGENINGEN AND COLOGNE

Although at this stage in my career I had an active program of research funded by grants, I had the feeling that it would not be easy to get funding in the future. This was partly because Wageningen University around 2000 was not in a good shape, with decreasing student numbers and staff reductions, and partly because it seemed that I was not eligible for the large personal grants that were given out by the Dutch funding agencies. For some programs I was too established and for others apparently not suitable. When I received a telephone call in 2003 inquiring if I was interested in applying for a directorship at the Max Planck Institute (MPI) for Plant Breeding Research in Cologne, I decided it was the right time to move. I knew colleagues in two German MPI institutes who were part of the Natural program, and I was also a member of the Scientific Advisory Board of the Cologne and Golm MPIs. The latter had given me an insight into the way such institutes functioned. This position also gave me the possibility to follow up research lines that were more difficult to pursue in Wageningen, especially the work on seed dormancy, where Leónie Bentsink had just cloned *DOG1* (9). We also had several mutants and QTLs prepared (mapped) for future cloning.

Within the Max Planck system, as I saw it, I would work on these topics with young group leaders, although I could also run a group myself. I decided that my own group should mainly work on resource development and most of the department budget should go to the groups within the department. This included some outside grants that I was able to acquire. I did not start with PhD students supervised only by myself because I assumed I was not the right person to supervise people on a daily basis in molecular biology, since I had never practiced it myself. I also asked the Max Planck Society and Wageningen University to keep a one-day-per-week position in Wageningen for me, in order to stay connected and to keep the links with Wageningen groups. It enabled me to complete the supervision of the PhD students working in Wageningen, as I was still formally the head of the botany section in the genetics laboratory. I also had to act as the formal PhD supervisor of PhD students working with Mark Aarts and Hans de Jong until they became full professors. A full professorship is a requirement to be an official PhD supervisor (called a promotor) in the Netherlands. This meant that I read manuscripts and discussed their projects. We also kept our house in Wageningen since family and many friends were living in the Netherlands and the distance was less than 200 km from Cologne. The promotion of the interaction between Wageningen and Cologne has worked well. Know-how on computational genetics was lacking in Cologne but present in Wageningen and could be provided by a postdoc and the group of Professor Fred van Eeuwijk. Cologne became a useful addition for some Wageningen projects, especially when using the genome facilities and doing collaboration on bioinformatics. Examples of such interaction resulted in good papers (8, 47), and they include several more papers where sometimes I was a coauthor and sometimes just a mediator.

Getting involved in the Max Planck organization and the plant science community in Germany was a very interesting experience. Two group leaders that I had known for many years were in my department, and I could appoint three new group leaders. The latter I asked to work on topics that could be considered as following up on work from Wageningen. These were Wim Soppe for seed biology and Matthieu Reymond for the genetics of physiological traits, and for a new topic in population genetics, I appointed Dr. Juliette de Meaux, who had done her postdoc at the MPI in

Jena, Germany. When Matthieu and Juliette found permanent positions elsewhere, I could appoint José M. Jiménez-Gómez and Ales Pecinka as group leaders, bringing in genomics and epigenetic expertise. I considered my own role mainly to develop resources and work on projects that were not picked up by the groups. It was clear that the young group leaders running their own research in an independent way needed to get enough visibility, since their positions were not permanent and they needed to apply for jobs after spending some time at an MPI. Being at a multidisciplinary institute and having excellent colleagues allowed interaction beyond the departments in a similar way to that in Wageningen. An example of this was the finding by Ruben Alcázar and Matthieu Reymond of incompatibility genes segregating in *Arabidopsis* populations that we had developed in Wageningen (4). Because it was clear that plant–pathogen interactions were involved in this phenomenon, a very fruitful collaboration was initiated between the groups of Matthieu and Jane Parker. Near the end of my time in Cologne, I realized that I missed interaction with PhD students and the detailed discussions with them. Therefore, I appointed three PhD students with whom I was involved in daily supervision. I realized that the difference between the Dutch and German plant science community was mainly size and I felt that I did not have as many interactions in Germany compared to the experience in the Netherlands. However, since I was no longer involved in recent Dutch programs, I also felt that I had lost contacts there. I had never liked a lot of traveling and committee work, and my own research had probably become less visible. However, seeing a younger generation following and developing lines of research that I was earlier involved in was very satisfying, and I still have contacts with many of these groups and former colleagues, even after my retirement at the end of 2015.

I realize that during my career I was part of one of the most exciting periods in plant science, in which plant biology became one topic instead of a group of multiple disciplines. The progress in finding the molecular and biochemical basis of many processes was impressive, mainly due to the application of molecular biology. The basis of this was often the use of genetic variation, and this was where I contributed: first with the isolation and preliminary characterization of several important mutant groups and later by adding methods and materials to the study of natural variation. Things became even more exciting with new developments in sequence technology and the links with the fields of ecology and evolution.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

I wish to thank my teachers, colleagues, collaborators, and students, including the many not mentioned in this article, for making it possible for us to contribute to the development of plant research. I am particularly grateful for the many exciting and rewarding scientific collaborations I have had during my career. I thank Machi Dilworth (National Science Foundation) who provided me with details on the history of the *Arabidopsis* genome project. I also thank Carlos Alonso-Blanco, Wim Soppe, Marcel Dicke, and Chris and Dick Kendrick for reading the drafts of the manuscript.

LITERATURE CITED

1. 1001 Genomes Consort. 2016. 1,135 genomes reveal the global pattern of polymorphism in *Arabidopsis thaliana*. *Cell* 166:481–91
2. Aarts MGM, Dirkse WG, Stiekema WJ, Pereira A. 1993. Transposon tagging of a male sterility gene in *Arabidopsis*. *Nature* 363:715–17

3. Ahmad M, Cashmore AR. 1993. *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. *Nature* 366:162–66
4. Alcázar R, García AV, Parker JE, Reymond M. 2009. Incremental steps toward incompatibility revealed by Arabidopsis epistatic interactions modulating salicylic acid pathway activation. *PNAS* 106:334–39
5. Alonso-Blanco C, Blankestijn-de Vries H, Hanhart CJ, Koornneef M. 1999. Natural allelic variation at seed size loci in relation to other life history traits of *Arabidopsis thaliana*. *PNAS* 96:4710–17
6. Alonso-Blanco C, Koornneef M. 2000. Naturally occurring variation in *Arabidopsis*: an underexploited resource for plant genetics. *Trends Plant Sci*. 5:22–29
7. *Arabidopsis* Genome Initiat. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408:796–15
8. Assunção AGL, Herrero E, Lin Y-F, Huettel B, Talukdar S, et al. 2010. *Arabidopsis thaliana* transcription factors bZIP19 and bZIP23 regulate the adaptation to zinc deficiency. *PNAS* 107:10296–301
9. Bentsink L, Jowett J, Hanhart CJ, Koornneef M. 2006. Cloning of *DOG1*, a quantitative trait locus controlling seed dormancy in *Arabidopsis*. *PNAS* 103:17042–47
10. Debeaujon I, Léon-Kloosterziel KM, Koornneef M. 2000. Influence of the testa on seed dormancy, germination and longevity in Arabidopsis. *Plant Physiol*. 122:403–14
11. El-Assal SED, Alonso-Blanco C, Peeters AJM, Raz V, Koornneef M. 2001. A QTL for flowering time in *Arabidopsis* reveals a novel allele of *CRY2*. *Nat. Genet.* 29:435–40
12. Giraudat J, Hauge BM, Valon C, Smalle J, Parcy F, Goodman HM. 1992. Isolation of the Arabidopsis *ABI3* gene by positional cloning. *Plant Cell* 4:1251–61
13. Jansen RC, Van Ooijen JW, Stam P, Lister C, Dean C. 1995. Genotype-by-environment interaction in genetic mapping of multiple quantitative trait loci. *Theor. Appl. Genet.* 91:33–37
14. Jensen J, Jørgensen JH. 1975. The barley chromosome 5 linkage map. II. Extension of the map with four loci. *Hereditas* 80:17–26
15. Jongsma M, Koornneef M, Zabel P, Hille J. 1987. Tomato protoplast DNA isolation: physical linkage and recombination of exogenous DNA sequencies. *Plant Mol. Biol.* 8:383–89
16. Karssen CM. 2002. Germination, dormancy and red tape. *Seed Sci. Res.* 12:203–16
17. Karssen CM, Brinkhorst-van der Swan DLC, Breekland AE, Koornneef M. 1983. Induction of dormancy during seed development by endogenous abscisic acid: studies on abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.) Heynh. *Planta* 157:158–65
18. Keurentjes JJB, Fu J, de Vos CHR, Lommen A, Hall RD, et al. 2006. The genetics of plant metabolism. *Nat. Genet.* 38:842–49
19. Koncz C. 2006. Dedication: George P. Rédei: *Arabidopsis* geneticist and polymath. *Plant Breed. Rev.* 26:1–23
20. Koornneef M. 1981. The complex syndrome of *ttg* mutants. *Arabidopsis Inf. Serv.* 18:45–51
21. Koornneef M, Barbaro A, van der Veen JH. 1977. Nongerminating, gibberellic acid responsive mutants in *Arabidopsis thaliana*. *Arabidopsis Inf. Serv.* 14:14–17
22. Koornneef M, Cone JW, Dekens RG, O'Herne-Robers EG, Spruit CJP, Kendrick RE. 1985. Photomorphogenic responses of long hypocotyl mutants of tomato. *J. Plant Physiol.* 120(2):153–65
23. Koornneef M, Hanhart CJ. 1984. The localization of two ABA-insensitivity genes. *Arabidopsis Inf. Serv.* 21:5–10
24. Koornneef M, Hanhart CJ, Jongsma M, Toma I, Weide R, et al. 1986. Breeding of a tomato genotype readily accessible to genetic manipulation. *Plant Sci*. 45:201–8
25. Koornneef M, Hanhart CJ, van der Veen JH. 1991. A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Mol. Gen. Genet.* 229:57–66
26. Koornneef M, Meinke DW. 2010. The development of Arabidopsis as a model plant. *Plant J.* 61:909–21
27. Koornneef M, Reuling G, Karssen CM. 1984. The isolation and characterization of abscisic acid-insensitive mutants of *Arabidopsis thaliana*. *Physiol. Plant.* 61:377–83
28. Koornneef M, Rolff E, Spruit CJ. 1980. Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. *Z. Pflanzenphysiol.* 100:147–60
29. Koornneef M, van Diepen JAM, Hanhart CJ, Kieboom-de Waart AC, Martinelli L, et al. 1989. Chromosomal instability in cell- and tissue cultures of tomato haploids and diploids. *Euphytica* 43:179–86

30. Koornneef M, van Eden J, Hanhart CJ, DeJongh AMM. 1983. Genetic fine-structure of the *GA-1* locus in the higher plant *Arabidopsis thaliana* (L.) Heynh. *Genet. Res.* 41:57–68
31. Koornneef M, van Eden J, Hanhart CJ, Stam P, Braaksma FJ, Feenstra WJ. 1983. Linkage map of *Arabidopsis thaliana*. *J. Hered.* 74:265–72
32. Kubo H, Peeters AJM, Aarts MGM, Pereira A, Koornneef M. 1999. *ANTHOCYANINLESS 2*, a homeobox gene affecting anthocyanin distribution and root development in Arabidopsis. *Plant Cell* 11:1217–26
33. Laibach F. 1943. *Arabidopsis thaliana* (L.) Heynh. als Objekt für genetische und entwicklungsphysiologische Untersuchungen. *Botan. Arch.* 44:439–55
34. Leonelli S. 2007. *Arabidopsis*, the botanical *Drosophila*: from mouse cress to model organism. *Endeavour* 31:34–38
35. Magnien E, Bevan M, Planqué K. 1992. A European 'BRIDGE' project to tackle a model plant genome. *Trends Biotechnol.* 10:12–15
36. Meyerowitz EM. 2001. Prehistory and history of Arabidopsis research. *Plant Physiol.* 125:15–19
37. Parks BM, Shanklin J, Koornneef M, Kendrick RE, Quail PH. 1989. Immunochemically detectable phytochrome is present at normal levels but is photochemically nonfunctional in the *hy 1* and *hy 2* long hypocotyl mutants of *Arabidopsis*. *Plant Mol. Biol.* 12:425–37
38. Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, et al. 1999. 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* 400:256–61
39. Provart NJ, Alonso J, Assmann SM, Bergmann D, Brady SM, et al. 2016. 50 years of Arabidopsis research: highlights and future directions. *New Phytol.* 209:921–44
40. Somerville C. 1989. *Arabidopsis* blooms. *Plant Cell* 1:1131–35
41. Somerville C, Koornneef M. 2002. A fortunate choice: the history of *Arabidopsis* as a model plant. *Nat. Rev. Genet.* 3:883–89
42. Soppe WJ, Jacobsen SE, Alonso-Blanco C, Jackson JP, Kakutani T, et al. 2000. The late flowering phenotype of *fwa* mutants is caused by gain-of-function epigenetic alleles of a homeodomain gene. *Mol. Cell* 6:791–802
43. Stam P. 1993. Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. *Plant J.* 3:739–44
44. Sun T, Goodman HM, Ausubel FM. 1992. Cloning the Arabidopsis *GA1* locus by genomic subtraction. *Plant Cell* 4:119–28
45. van der Schaar W, Alonso-Blanco C, Léon-Kloosterziel KM, Jansen RC, van Ooijen JW, et al. 1997. QTL analysis of seed dormancy in *Arabidopsis* using recombinant inbred lines and MQM mapping. *Heredity* 79:190–200
46. van Wordragen MF, Weide RL, Coppoolse E, Koornneef M, Zabel P. 1996. Tomato chromosome 6: a high resolution map of the long arm and construction of a composite integrated marker-order map. *Theor. Appl. Genet.* 92:1065–72
47. Wijnker E, Velikkakam James G, Ding J, Becker F, Klasen JR, et al. 2013. The genomic landscape of meiotic crossovers and gene conversions in *Arabidopsis thaliana*. *eLife* 2:e01426
48. Zeevaart JAD. 2009. My journey from horticulture to plant biology. *Annu. Rev. Plant Biol.* 60:1–19