Paulina Tomaszewska¹,²), Romuald Kosina²*)

Variability of pollen grains quality in oat amphiploids and their parental species

¹)Department of Genetics and Genome Biology, University of Leicester, United Kingdom
²)Faculty of Biological Sciences, University of Wrocław, Poland

*)Correspondence: R. Kosina, Faculty of Biological Sciences, University of Wrocław, Przybyszewskiego 63, 51-148 Wrocław, Poland; e-mail: romuald.kosina@uwr.edu.pl;
PT orcid.org/0000-0002-9596-7219
RK orcid.org/0000-0001-5185-416X

Abstract

The reproductive potential of oat species and their hybrid progeny (amphiploids) was evaluated during three vegetation seasons. Morphotypes and viability of pollen grains were described by means of correlations and regression, while relationships between taxa were analysed with the use of numerical taxonomy methods. Size of pollen grains varied between the growing seasons, but the relations between the taxa appeared to be stable. Viability of pollen grains was environmentally modified and showed no correlation with pollen length. In an ordination space, amphiploids were discriminated from parental species. In both group of
plants, a positive correlation between the pollen size and the level of ploidy was maintained; however, along a regression line, amphiploids were located among species with a high level of ploidy and were extreme units deviating from the regression line. Developmental anomalies of pollen grains had a low frequency, with the formation of micrograins being the most common event. Such a pattern of development can prove that some pollen grains were chromosomally unbalanced. Anomalous morphotypes of pollen were more common in hybrid types than in species, including pollens with many poruses, which were found only in amphiploids. Frequencies of multiporate grains and micropollens were strongly correlated. In an ordination space, monoporate types (species) were discriminated from multiporate types (amphiploids). In general, the high level of pollen viability in amphiploids can prove their genomic stabilisation through many generations of their reproduction.

**Keywords** Avena · Amphiploids · Species · Pollen developmental anomalies · Pollen quality · Correlation · Regression · Numerical taxonomy

**Introduction**

Research on the development and variability of pollen grains is important for understanding the mating system in plants, including their common hybrid swarms. The variability of the mating systems influences the variability and the differentiation processes observed in the populations of wild and cultivated plants (Grant 1981; Richards 1986). In cereals, the most important grasses to mankind, both natural and/or artificial selections through subsequent generations stabilise a reproduction process at the even number of chromosomes (Kushwaha et al. 2004). Such changes also apply to wild and cultivated oats as well as their artificial hybrids.
Reproductive success depends primarily on the pool of viable pollen grains available during the flowering time. This is ensured by regular meiosis, typical for fixed taxa; however, in some species, but especially in hybrids, this process can be irregular. Various anomalies related to chromosomes or activity of karyokinetic spindle were observed in meiosis of some Avena L. species (Sheidai et al. 2003), cultivars of Avena sativa L. (Baptista-Giacomelli et al. 2000a, b) or Urochloa decumbens (Stapf) R.D.Webster (previously classified as Brachiaria decumbens Stapf; Mendes-Bonato et al. 2002). These anomalies often ended with the formation of cytoplasmic separations with micronuclei and ultimately micropollens. Meiotic disturbances were more severe and more frequent in interspecific and intergeneric hybrids (Mujeeb et al. 1978; Linde-Laursen and von Bothmer 1993; Li et al. 2005); however, their pattern was similar. Thus, studies on the frequency of micronuclei and micropollens can be a good tool for evaluation of the reproductive quality of the plant. Meiotic disorders can also be found even in one anther sac, where the microsporocyte mother tissue forms a mosaic of cells with different numbers of chromosomes. Such an event was found in the amphiploid Avena abyssinica × A. strigosa (Thomas and Peregrine 1964) and in Allium senescens subsp. montanum (Pohl) Holub (synonym of accepted Allium senescens L.) (Małecka 2008).

In the highly polyploid species or hybrids, including amphiploids with a doubled chromosome set, a change from generative into apomictic reproduction was described (Grant 1981; Quarin et al. 2001; Ma et al. 2009). A very special morphogenesis of pollen grains in the form of multiporate units has been interpreted as related to apomixis (Ma et al. 2009). Multiporate grains appeared to be ineffective due to the germination of pollen tubes through several pores and their mutual competition under a tube growth (Florek 2013; Kosina et al. 2014). A pattern of porus formation is probably determined by metabolism and deposition of callose before a porus morphogenesis (Teng et al. 2005). It was also evidenced that the callose deposition in a pollen grain varied among grasses (Warzych 2001; Kłyk 2005).
Evaluation of the reproductive stability of plant hybrids and their relatives is important for breeding purposes. Therefore, this article presents comparative analyses of pollen quality in oat species showing a broad ploidy spectrum and their amphiploids. Some components of the mating system of oat species and amphiploids were assessed taking into account the morphology and viability of pollen grains as indicators of hybrid vigor.

**Materials and methods**

Oat amphiploids and their parental species, listed in Table 1, were cultivated on small plots in the grass collection (Wrocław, SW Poland) maintained by R. Kosina. During the plot experiments, the plants were grown under the same soil-climatic conditions. Mature stamens were sampled from plants at the turn of June and July, for the three consecutive growing seasons. Each year, mature stamens taken from 5 plants of each of the 15 accessions (OTUs, Operational Taxonomic Units) were analysed. Stamens were always collected from the lower flower of spikelet. Pollen grains isolated from anther were placed on microscope slides, stained with 0.5% v/v acetocarmine or 0.01% v/v acridine orange, mounted in glycerin, and covered with a coverslip. The botanical nomenclature of studied species was applied according to https://npgsweb.arsgrin.gov/gringlobal/taxon/taxonomysearch.aspx, accessed on 21 July 2021, and http://www.theplantlist.org/, accessed on 21 July 2021:

- *A. fatua* L.
- *A. sterilis* L.
- *A. sativa* L.
- *A. barbata* Pott ex Link
- *A. abyssinica* Hochst.
- *A. magna* H.C. Murphy & Terrell
- *A. strigosa* Schreb.
A. longiligumis Durieu

A. eriantha Durieu

**Microscopy**

The slides were examined under an Olympus BX-60 epifluorescence microscope (Hamburg, Germany), using both white and UV light. Images were taken with an Olympus E-520 camera (Olympus Imaging Europe, Hamburg, Germany).

**Statistical analysis**

OTUs have been described by the following characteristics of pollen grains:

- viability (all viable, dysfunctional and dead pollen grains taken from anther were counted),
- length of the pollen grain (measured for forty randomly selected pollen grains from anther),
- impaired pollen grains (all micrograins, unreduced pollen grains, and grains of irregular shape were counted),
- number of poruses in pollen grains.

The OTUs were arranged into an ordination space with application of non-metric multidimensional scaling (Kruskal 1964; Rohlf 1981) and according to Rohlf’s numerical approach (Rohlf 1994). The correlation and regression analyses were made according to KWIKSTAT 4 procedure (Elliot 1994).

**Results**

**Viability and size of pollen grains**

The viability of the pollen grains was determined using the acetocarmine staining technique, which allowed the grains to be classified into three groups:

- normal, viable pollen - stained red, completely filled with cytoplasm,
dysfunctional, defective pollen - weakly coloured, partially filled with cytoplasm,
empty, dead pollen - uncoloured, with no cytoplasm, usually wrinkled or cracked.

Defective and dead pollen grains were treated together as non-viable pollens in statistical analyses.

Table 2 presents the results of analyses concerning the percentage of viable pollen grains in the examined accessions, taking into account the three consecutive growing seasons. Accessions of both amphiploids and their parental species were characterised by a high level of pollen grain viability. The average percentage of viable pollen for three years was over 90% in all examined accessions. Amphiploids showed a slightly lower viability of pollen grains compared to their parental species. The percentage of viable pollen in species ranged from 96.3 to 98.9%, and in amphiploids the range was 90.5 - 96.0%. Octoploid amphiploids were characterised by the lowest pollen viability: the average percentage of pollen completely filled with cytoplasm in b/sn and e/sa was 91.1% and 90.5%, respectively. In some growing seasons, some species and amphiploids showed pollen grain viability below 90% or pollen was not obtained for analysis due to problems with seed germination or premature drying of plants due to the drought. Over the three-year period, the range of variation of pollen viability between the three growing seasons was greatest in the amphiploid f/ste (from 82.7 to 98.0%) and the hexaploid species Asa (from 88.5 to 99.0%).

The amphiploids and their parental species varied in the pollen size. Oat pollen grains were usually slightly elongated, elliptical in shape. Thus, the measurements of their length were sufficient to describe size of pollen grains, and these data are included in Table 2. The average length of grains ranged from 37.6 to 53.9 µm. The smallest grains were observed in Ae, while the largest pollen was found in the octoploid e/sa. The mean length of the elliptical grains in the amphiploid e/sa was over 50 µm in all years. The size of pollen grains varied not only between the analysed accessions, but also between years. The latter depended on the
weather conditions, such as temperature and water supply, during the development of pollen grains.

The Pearson’s correlation coefficients matrix of the pollen grain characteristics (pollen viability and their average length in particular growing seasons) is presented in Table 3. The average length of pollen grains in three consecutive growing seasons were correlated with each other. The significant correlations of the pollen grain size between different growing seasons mean that this feature was a stable characteristic of the analysed taxa. The lack of correlation between the viability of pollen grains in individual years indicated a random, non-directional, high environmental variation of this feature. The matrix of the Pearson’s correlation coefficients for pollen grain characteristics was used to distribute the fifteen studied oat accessions (OTUs) in an ordination space along three axes (x, y, z) using the Kruskal's non-metric multidimensional scaling (nmMDS). The diagram of the minimum spanning tree (MST) (Fig. 1) shows the location of OTUs connected to each other by the smallest links. The smallest distance was observed between the OTUs Aa and Ab. The location of Asa in the vicinity of the taxon with small pollen grains is probably conditioned by the inter-variety variability of this cultivated species. The extreme position of the amphiploid e/sa was mainly determined by its largest pollen grains. In the diagram, a large distance between the two amphiploids e/sa and f/ste, which showed similar viability of pollen grains in different growing seasons, was due to the different size of pollen grains in both taxa. The diagram also shows a certain directional difference between species and their progeny - the species formed a group located in the center of the diagram, while amphiploids were scattered outside (Fig. 1). This means that a pattern of seasonal variation of two analysed characteristics varied between oat species and amphiploids; however, this was not complete discrimination as Aa and Ae appeared to be outside of the center.
Regression of pollen length versus ploidy

The research showed a significant correlation between the length of pollen grains and the level of ploidy provided for the root tissues (Table 1; P. Tomaszewska pers. comm.). The value of the Pearson’s correlation coefficient for a random sample of $n=15$ OTUs was $r=0.78$, which indicates the significant regression relationship between the variables (Fig. 2). Low values of both characteristics were observed in di- and tetraploid species, while hexaploid species and amphiploids were located in the right part of the diagram showing higher values. In addition, for the extreme OTUs, $e/sa$ and $b/sn$, a high frequency of multiporate pollen grains was noted (see Tables 5 and 6).

Anomalous pollen grains

Apart from normal pollen grains, some grains showed developmental disorders, and their examples are presented in Fig. 3. In all the examined accessions, pollen micrograins were observed (Fig. 3a), but their percentage share in the entire pool of pollen grains tested did not exceed 1% (Table 4). $Astr$, as well as three amphiploids ($e/sa$, $m/l$ and $a/str$), showed very large, unreduced pollen grains. Some such grains are shown in Fig. 3b,d,e,f. Their considerable size may prove a higher level of ploidy of pollen stem cell or non-reduction of gametes. It may also be caused by disturbances in the formation of cell walls during subsequent cytokineses in the microsporogenesis process, which was confirmed by the irregular shape and depressions in the surface of some large pollen grains (Fig. 3c,g,h). However, the frequency of large pollen grains was low and did not exceed 1%. In some cases, the irregular shape of the pollen grains suggests an asymmetric division that led to the formation of micropollens (Fig. 3j). In the amphiploid $e/sa$, a single case of a huge, highly elongated pollen was recorded (Fig. 3k). It was estimated to be about 4-5 times larger than a normally developed grain, and had seven poruses, including two connected. Anomalous pollen grains were observed in all amphiploids, but also in one hexaploid and three tetraploid
species (Table 4). However, they constituted a small percentage of the total pollen pool analysed. The highest amount of pollen of irregular shape was observed in octoploids, but their frequency was only 0.06% and 0.05% in e/sa and b/sn, respectively. Micrograins appeared to be most common among other anomalies. Their average frequency was in amphiploids 0.51%, while in the parental species it was distinctly lower 0.21%.

**Number of poruses in cell walls of pollen grains**

As shown in Fig. 3, irregularly shaped pollen grains often had more than one porus. The analysis of the number of poruses was performed under UV light after staining the pollen with acridine orange. Normal pollen grains of the analysed oat accessions had one porus (Fig. 4a). However, in all tested amphiploids, also in their normal pollen grains, two poruses were observed sporadically. This phenomenon concerned only amphiploids and was absent in the parental species. The position of the poruses in relation to each other was different. Sometimes they were distant (Fig. 4c) or close to each other (Fig. 4e), and in some cases connected to each other (Fig. 4b,d). The diameters of both poruses were similar or different (Fig. 4c,f). In the amphiploid b/sn, single cases of pollen with three poruses (Fig. 4f) and four poruses were reported. Amphiploid e/sa also had pollen grains with a greater number of poruses. An extreme case of the multiporate grain was noted in this amphiploid (Fig. 3k). Pollen with three and four poruses were not observed in the remaining four amphiploids. Table 5 shows that the frequency of pollen grains with an increased number of poruses was low. b/sn showed the highest frequency of pollen grains with many poruses in comparison to other amphiploids.

**Correlation between pollen grain characteristics**

For all the pollen characteristics (arithmetic averages from three growing seasons), the Pearson’s correlation coefficient matrix was calculated. Table 6 shows that the viability of pollen grains was positively correlated only with pollen grains having one porus. There were
also negative correlation coefficients between pollen viability and all other characteristics. This means that taxa which produce micropollens, non-reduced pollen grains, irregular pollen and multiporate grains will show a lower ability to germinate pollen grains. Disturbances in pollen development significantly affect its viability. The viability of the pollen was also negatively correlated with the length of the pollen grains. In plants with a higher level of ploidy, and thus having larger pollen grains, a reduced pollen viability can be observed comparing to diploid species producing haploid pollen grains. The presence of micrograins was positively correlated with irregular, three- and four-porus pollen, and negatively correlated with one-porus pollen. Moreover, a positive correlation was demonstrated between the presence of pollen with more than four poruses and the length of pollen grains and non-reduced pollen. Other positive correlations occurred between irregular pollen and the length of the pollen grains, as well as between multiporate pollen grains. On the other hand, the presence of irregular pollen was negatively correlated with one-porus grain. When analysing the relationships between the characteristics of pollen grains related to the number of poruses, a negative correlation was noted between one-porus pollen and pollen with two, three and four poruses. The development of two-, three- and four-porus grains was positively correlated. All these data clearly show that the larger the pollen, and thus the higher the level of ploidy, the more disturbances in microsporogenesis, leading to the formation of anomalous pollen grains.

The Pearson’s correlation coefficient matrix of ten pollen grain characteristics (Table 6) was used to distribute the fifteen studied oat accessions (OTUs) in the space of three ordination axes using the Kruskal's non-metric multidimensional scaling (nmMDS). A diagram of the minimum spanning tree (MST) (Fig.5) shows the location of the OTUs. The nmMDS analysis revealed significant variability between the taxa studied here. Diploid Ae and two octoploids b/sn and e/sa occupy the opposite positions in the MST diagrams. The
position of $Ae$ in the diagram was determined by the very small size of pollen grains, as well as the complete absence of non-reduced and irregular pollen grains in the analysed pollen pool from three growing seasons. The other two diploids, $Al$ and $Astr$, also showed no significant disturbances in the development of pollen grains, but it was the size of the pollen grains that influenced the placement of the OTUs in diagram. $Astr$ was located close to the tetraploid species $Am$ and $Aa$. In turn, $Al$ showed similarity in the values of the studied traits to the amphiploid $b/sa$. The phenotypic distance between these two OTUs was very small. The second extreme group in MST diagrams was created by octoploids. Their location in the ordination space was due to the slightly reduced viability of pollen grains, as well as the higher frequency of irregular pollen grains compared to the other studied accessions. Moreover, both extreme amphiploids ($b/sn$, $e/sa$) were distinguished from other taxa by the higher frequency of multiporate pollens. Despite this, there was a clear discrimination between both OTUs. It may result from significant differences in the size of pollen grains between the accessions of the studied species and amphiploids. The length of the pollen grains was greatest in the amphiploid $e/sa$. In turn, $b/sn$, despite the same level of ploidy as $e/sa$, produced pollen of a size comparable to hexaploid amphiploids. Such a distribution of both OTUs could also be influenced by the differences in the percentage share of micropollens and non-reduced pollen grains in the studied pools. The small distance between $Ab$ and $Astr$ indicates their phenotypic similarity of the analysed traits. The viability of pollen grains did not differentiate them from other studied accessions, but both species were characterised by the production of pollen of a similar size. It is clear that mono- and multiporate taxa (species versus hybrid units) were well discriminated in the diagram.

**Discussion**

The pollen variability (Dzyuba et. al. 2006; Karabournioti et. al. 2007; Costa et al. 2016) can be evaluated by measuring pollen viability, assessing its morphology, and studying pollen-
stigma compatibility reactions, thus obtaining important information on the plant breeding systems (Kosina and Tomaszewska 2015). Other biological parameters, such as pollen size (De Storme et al. 2013) and number of poruses (Kihara 1982; Kalinowski et al. 2001, 2005), are important for assessing the ploidy of plants, in particular of newly formed hybrid units.

**Size of pollen grains**

The lengths of the pollen grains were provided for some species and amphiploids of oat (Katsiotis and Forsberg 1995b; Meo 1999; Chrzątek et al. 2009). Among the accessions studied here, the largest pollen grains were found in an octoploid *A. eriantha* × *A. sativa*, but some hexaploid species differed from values published by Katsiotis and Forsberg (1995b). The differences may be related to the inter-cultivar variation, or the influence of environmental conditions on the size of the pollen grains. The variation of pollen size between seasons can confirm that external factors influenced this characteristic; however, a pattern of inter-OTU differences was maintained (see Table 2, 3).

The size of the pollen is determined by the ploidy level and genotypes of both the gametophyte and the sporophyte in which the pollen grains are produced (Ottaviano and Mulcahy 1989; Southworth and Pfahler 1992; Katsiotis and Forsberg 1995b). Our research on fifteen oat accessions showed a statistically significant correlation between ploidy and length of pollen grains. According to Katsiotis and Forsberg (1995b), the size of pollen grains was determined primarily by the level of ploidy, and only to a small extent by interspecific variation at the same level of ploidy. They proved that there were no significant differences in pollen size between diploid and tetraploid accessions of oats and between hexaploids and octoploids. However, Southworth and Pfahler (1992) found that the size of pollen was influenced not only by the level of ploidy, but also by the number of genomes and volume of cytoplasm, and interactions between these variables. The development of pollen grains in the pollen sac may also be significantly influenced by the orientation of cytokineses in the
microspore stem cell and the microspore - tapetum spatial relationship (Kosina and Florek 2011). In grasses, microsporocytes are arranged in one layer which adheres to tapetum (Bhandari 1984; Batygina 1987). Such an architecture of the anther sac provides the equal nutritional support for microspores formed from microsporocytes after two anticlinal cytokineses. Looking from outside into the anther sac, a uniform mosaic of isolateral tetrads of microspores can be seen (Batygina 1987). However, if cytokineses are a combination of anticlinal and periclinal events or there are no cytokineses, so microspores within a tetrad are not equally nourished by tapetum (Brown and Lemmon 2000; Kosina and Florek 2011). Such development creates microspores and pollen grains of various size and mating value. Thus, the large, normal and small microspores form a mosaic at walls of the anther sac. The mosaics were observed in various plant organs (Kosina 2007; Tomaszewska and Kosina 2018), and also in anthers of Allium senescens subsp. montanum (Małecka 2008). In addition, natural mutations or transgressive segregation may occur in the pollen sac in the genus Avena, broadening the old by new variability (Kosina 2015).

**Pollen viability**

Pollen grains are considered viable when they are able to germinate on the stigma. There are many different methods for determining pollen viability, and their advantages and disadvantages have been discussed by Dafni and Firmage (2000). The authors suggested the use of several tests simultaneously, but the results obtained by Platje (2003) confirm the effectiveness of simple and quick in vivo pollen staining with acetocarmine.

Factors influencing the viability of pollen grains include flower morphology, environmental conditions (temperature, humidity, soil conditions, nitrogen availability, seasonality) and indirect factors (pollen metabolism, number of nuclei, genetic conditions, breeding methods) (Dafni and Firmage 2000). The differences in pollen viability between consecutive growing seasons suggest the influence of external factors on this trait (see Tables
and 3). Some amphiploids and parental species studied here showed a reduced viability of pollen grains in one of the growing seasons, which was most likely caused by unfavorable weather conditions, such as high temperature or air humidity. It is known that the early stages of microspore stem cell development are particularly sensitive to high temperature (Sakata et al. 2000). Some of the fifteen examined oat taxa could be particularly sensitive to weather conditions, which resulted in a reduced viability of pollen grains in some growing seasons.

Our studies showed that the percentage of viable pollen was slightly lower in oat amphiploids compared to their parental species. Analysis of pollen viability in *A. fatua* and *fatuos* carried out by Warzych (2001) indicated a different percentage of viable pollen between accessions, plants from one accession, and between flowers located in one spikelet. Significant information on the viability of pollen grains was provided by studies on the reproductive system of other grass hybrids. The frequency of viable pollen was significantly lower in hybrids involving *Agrostis* L. and *Polypogon* Desf. compared to the parental species (Zhao et al. 2007). The average pollen viability of interspecific hybrids was higher than that of intergeneric hybrids. A similar tendency was shown by amphiploids resulting from the crossing of *Aegilops ovata* L. with *Secale cereale* L. (Wojciechowska and Pudelska 2002) and *Aegilops kotschyi* Boiss. and *Aegilops biuncialis* Vis. with *S. cereale* (Wojciechowska and Pudelska 2005). However, oat amphiploids appear to be genetically stable and meiotically compatible (Loskutov 2001) despite having distant genomes that exhibit numerous intergenomic translocations (Tomaszewksa and Kosina 2018, 2021). The above phenomenon can be explained by the selection shift of the chromosome number, e.g. in the *Avena sativa × Avena maroccana* Gand. (*A. maroccana* is a synonym of accepted *Avena magna* H. C. Murphy & Terrell) amphidecaploid (Kushwaha et al. 2004). In this amphidecaploid, the loss of chromosomes over many generations, and simultaneously stable cytotypes with the even number of chromosomes were formed.
Anomalous pollen development

Reduced viability of pollen grains or even complete sterility, often resulted from anomalous stamen development (Leighty and Sando 1924; Protasevich 1984; Murai and Tsunewaki 1993; Kosina and Florek 2011). No anomalous development of stamens was found in the oat accessions analysed here. Therefore, the observed variability in the viability of pollen grains between the accessions of amphiploids and their parental species may be caused by disturbances in meiosis resulting from the coexistence of several different genomes in the hybrid nuclei (Chen et al. 1977; Tomaszewska and Kosina 2021). Anomalous meiosis can lead to the formation of gametes with a reduced or increased number of chromosomes. Numerous disturbances in the meiotic division, such as delayed chromosomes in telophase I and precocious chromosome segregation, were observed in interspecific and intergeneric hybrids involving Agrostis and Polypogon (Zhao et al. 2007). The presence of micronuclei and formation of polyads including micropollens were found during microsporogenesis in various young hybrids or amphiploids: Triticum aestivum L. × Agropyron glaucum Roem. & Schult. (syn.) (Cicin 1978), Triticum turgidum L. × Aegilops squarrosa L. (synonym of accepted Aegilops tauschii Coss) (Kihara 1982), Triticum aestivum × Leymus mollis (Trin.) Pilg. (Li et al. 2005). Disturbances in meiosis were also observed in interspecific oat hybrids (Paczos-Grzęda 2003) and some oat species, including diploids (Sheidai et al. 2003). These include chromosomes delayed in anaphase I and II and telophase I and II, micronuclei present in the microspore tetrads, bridges, chromosome elimination, multipolar systems and cytomixis. Examples of similar disorders came from the meiotic analysis of the Zea mays L. inbred lines (Caetano-Pereira and Pagliarini 2001) in which premature chromosome segregation, delayed chromosomes and micronuclei have been observed. The participation of such disturbed gametes in fertilization may contribute to the formation of an aneuploid generation with reduced fertility and viability (Zhao et al. 2007).
Numerous examples of anomalous pollen grains can be found in the literature. Micropollens and ultra-micropollens seem to be quite common in oats (Kosina and Florek 2011), and may arise through asymmetric segregation of chromosomes (Baptista-Giacomelli et al. 2000). Irregular pollen grains can be formed by abnormal pollen wall formation, unfinished cytokinesis, and cytomixis. Similar disorders were observed in an amphiploid Triticum aestivum x Leymus mollis (Li et al. 2005). Many cytomixis cases were found in Diplotaxis harra (Forssk.) Bois., where aneuploid cells showed highly unequal number of chromosomes (Malallah and Attia 2003). Abnormal cell wall formation is one of the most common causes of large, non-reduced microspores (Ellison 1937). Anomalous cytokinesis may occur during mitosis or meiotic division I and II. In the hybrid A. barbata x A. strigosa subsp. hirtula (Lag.) Malzev (syn. of Avena strigosa Schreb), pollen binuclear stem cells were observed, the presence of which resulted from disturbances in cell wall formation during pre-meiotic mitosis (Holden and Mota 1956). The second nucleus, usually peripheral, underwent apoptosis preceded by chromatin fragmentation and nucleolus atrophy. Diploid gametes have also been observed in other species and hybrids of the genus Avena (Ellison 1937; Kosina and Florek 2011), and diploid gametes accounted for 1% of the pollen grains in tetraploid Avena vaviloviana Malzev (Mordv.) (Katsiotis and Forsberg 1995a). Large, unreduced microspores can be formed by cytomixis or restitution of nuclei during meiosis (Stuczyński et al. 1994). Unreduced pollen grains have low mating value and can be very ineffective in the fertilization process due to slow growth of their thick pollen tubes, as it was evidenced in Prunus spinosa L. (Staszak 2004).

**Number of poruses**

Morphogenesis of poruses seem to be related to de novo synthesis of callose in a microspore (Teng et al. 2005). In grasses, a pattern of callose deposition in a differentiating pollen grain appeared to be different. Scattered lenses of callose adjacent to intine were
observed in *Brachypodium phoenicoides* (L.) Roem. & Schult. (Klyk 2005), while in *Avena fatua* callose was synthesised around a pore and disappeared towards the opposite pole (Warzych 2001).

The number of poruses in the pollen wall, through which pollen tubes can germinate, varies between species. Pollens with different number of apertures often occur in the same species, including *Viola diversifolia* (DC.) W. Becker (Dajoz et al. 1991), *Viola arvensis* Murray and *Nicotiana tabacum* L. (Ressayre et al. 1998). The grass group is characterised by single-porate pollen grains, thus pollen with an increased number of poruses should be considered anomalous. Multiporate pollen grains were observed in all amphiploids studied here, while their parental species produced pollen with only one porus. Multiporate pollen grains were found in different hybrids and amphiploids of grasses: diporate grains were developed in an F1 hybrid generation of *Triticum turgidum* × *Aegilops squarrosa* (Kihara 1982), half of the pollen pool of *Miscanthus* Anderss. 'Giganteus' had two to five poruses (Linde-Laursen 1993), pollen with ten poruses has been reported in an amphiploid *Triticum aestivum* × *Leymus mollis* (Li et al. 2005). It should be emphasized that multiporate pollen grains can germinate through many pores. As a result, competition occurs between the tubes during their growth, which significantly reduces their effectiveness in the fertilization process. Such a case was observed in the amphiploid *Avena barbata* × *A. sativa* subsp. *nuda* (Florek 2013; Kosina et al. 2014).

The increase number of poruses may be caused by the independent activity of genes located in different genomes (Kalinowski et al. 2001, 2005). Therefore, plants formed by polyploidisation are often characterised by multiporate pollen grains. Some authors discuss the relationship between apomictic mode of reproduction and multiporate pollen. Liu et al. (2004) recorded multiporate pollen grains in *Leptochloa panicea* (Retz.) Ohwi and *Schmidtia pappophoroides* Steud. ex J.A.Schmidt, which showed apomictic type of reproduction. Other
apomicts among grasses that produce multiporate pollen include genera *Apluda* L., *Heteropogon* Pers., *Panicum* L., *Paspalum* L., *Pennisetum* Rich. (Guohua et al. 2009) and *Bothriochloa* Kuntze (Ma and Huang 2007). All of these genera include species showing high ploidy levels (Higgins et al. 2021; Tomaszewska et al. 2021a,b). It can be concluded that formation of multiporate pollen is associated with young allopolyploids and the interaction of various genomes in the nucleus, whereas apomictic reproduction allows the population to function with a generative defect. Allopolyploids often showed numerous disturbances in micro- and macrosporogenesis, which can lead to apomixis. In *Bothriochloa ischaemum* (L.) Keng, cytogenetic analyses revealed disturbed chromosome segregation and delayed chromosomes (Ma and Huang 2007). In *Calamagrostis hakonensis* Franch. & Sav. and *Dichanthium aristatum* (Poir.) C.E.Hubb (Guohua et al. 2009), double fertilisation was impossible due to sterility of pollen, thus apomixis was an alternative to sexual reproduction. It was found that a shift from the generative reproduction to apomixis occurred in plant populations showing higher numbers of chromosomes, where meiotic disorders and defective pollens appeared (Grant 1981). Thus, the interaction between the development of multiporate pollen grains and apomictic reproduction requires further research to understand what is the cause and what is the effect.

**Concluding remarks**

The pollen of the examined oat species and the amphiploids obtained from their crosses showed high viability, which can result in a proper development of caryopses. However, the pollen viability of amphiploids was several percent lower than in species. In addition, anomalous pollens were produced at low percent by the amphiploid flowers. It was found that the size of pollen grains varied during the growing seasons, but the interrelations between the studied taxa were stable. The viability of pollen grains was more significantly affected by the environment than the length of pollen grain and showed no correlation with the variability of
the grain size. In the ordination space, amphiploids were discriminated from parental species. In both groups of plants, a positive correlation was maintained between the pollen size and the level of ploidy, however, in the cloud of correlation points along the regression line, amphiploids were located among species with a high level of ploidy and were extreme units there. Developmental anomalies, shown in pollen morphotypes, had a low frequency, among them the formation of pollen micrograins was most common. Such an event proved that some pollen grains were chromosomally unbalanced and were formed in polyads. Anomalies were more common in the hybrid types, and the formation of pollen grains with many poruses occurred only in amphiploids and was strongly correlated with the frequency of micropollens. Multiporate grains will be less efficient for reproductive success due to their germination with many pollen tubes and mutual competition (Florek 2013; Kosina et al. 2014). In the ordination space, monoporate types (species) were well discriminated from multiporate types (amphiploids). The high level of pollen viability in amphiploids proved their probable genomic/chromosomal stabilisation through many generations of their reproduction in the life collections.

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Authors' contributions

PT designed and conducted all analyses and interpreted the results. PT and RK prepared figures and wrote the article. RK provided research idea, supervised the experiments and research documentation.

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References

Baptista-Giacomelli FR, Pagliarini MS, de Almeida JL (2000a) Elimination of micronuclei from microspores in a Brazilian oat (Avena sativa L.) variety. Genet Mol Biol 23:681-684

Baptista-Giacomelli FR, Pagliarini MS, de Almeida JL (2000b) Meiotic behaviour in several Brazilian oat cultivars (Avena sativa L.). Cytologia 65:371-378

Batygina TB (1987) Chlebnoe zerno. Atlas. Nauka, Leningrad

Bhandari NN (1984) The microsporangium. In: Johri B.M. (ed) Embryology of Angiosperms. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-69302-1_2

Brown RC, Lemmon BE (2000) The cytoskeleton and polarization during pollen development in Carex blanda (Cyperaceae). Amer J Bot 87:1-11
Caetano-Pereira CM, Pagliarini MS (2001) A new meiotic abnormality in Zea mays: multiple spindles associated with abnormal cytokinesis in both divisions. Genome 44:856-871

Chen C, Qualset CO, Stanford RH (1977) Meiotic studies of secondary 42-chromosome Triticales. Bot Bull Acad Sinica 18: 89-99.

Chrzastek M, Kruk K, Okoń S, Wojtowicz E (2009) Wpływ formy ojcowskiej na żywotność pyłku i niektóre cechy plonotwórcze mieszańców międzygatunkowych A. sativa L. cv. Borowiak x Avena sterilis L. Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin 252:245-253

Costa J, Castro S, Loureiro J, Barrett SCH (2016) Experimental insights on the function of ancillary pollen and stigma polymorphisms in plants with heteromorphic incompatibility. Evolution 71:121-134

Dafni A, Firmage D (2000). Pollen viability and longevity: practical, ecological and evolutionary implications. Plant Syst Evol 222:113-132

Dajoz I, Till-Bottraud I, Gouyon P-H(1991) Evolution of pollen morphology. Science 253:66-68

De Storme N, Zamariola L, Mau M, Sharbel F, Geelen D (2013) Volume-based pollen size analysis: an advanced method to assess somatic and gametophytic ploidy in flowering plants. Plant Reprod 26:65-81

Dzyuba OF, Shurekova OV, Tokarev PI (2006) On the natural polymorphism of pollen grains of Acer tataricum L. Paleontolog J 40:S590

Elliot AC (1994) KWIKSTAT 4- statistical data analysis program. Texasoft, Cedar Hill

Ellison W (1937) Polyploid gamete formation in diploid Avena hybrids. J Genet 34:287-295
Florek M (2013) Zmienność strukturalna i cytogenetyczna amfiploida *Avena barbata* × *Avena nuda* o genomach natywnych i demetylowanych. Dissertation, University of Wrocław.

Grant V (1981) Plant speciation. Columbia University Press, New York

Guohua M, Xuelin H, Qiusheng X, Bunn E (2009) Multiporate pollen and apomixis in Panicoideae. Pak J Bot 41:2073-2082

Higgins J, Tomaszewska P, Pellny TK, Castiblanco V, Arango J, Tohme J, Schwarzacher T, Mitchell RA, Heslop-Harrison JS, De Vega JJ (2021) The five *Urochloa* spp. used in development of tropical forage cultivars originate from defined subpopulations with differentiated gene pools. https://doi.org/10.1101/2021.07.21.453213

Holden JWH, Mota M (1956) Non-synchronised meiosis in binucleate pollen mother cells of an *Avena* hybrid. Heredity 10:109-117

Kalinowski A, Winiarczyk K, Wojciechowska B (2001) Pollen proteins after two-dimensional gel electrophoresis and pollen morphology of the amphiploids *Aegilops kotschyi* and *Ae. variabilis* with *Secale cereale*. Sex Plant Reprod 14:153-161

Kalinowski A, Klimko M, Wojciechowska B (2005) Pollen morphology and two-dimensional patterns of pollen coat and protoplast proteins in *Aegilops kotschyi* × *Secale cereale* amphiploids. Acta Biol Cracov Bot 47:97-110

Karabournioti S, Eleftheriou EP, Thrasyvoulou A, Fasseas C (2007) Pollen polymorphism in *Thymus capitatus* (Lamiaceae). Botany 85:493-500

Katsiotis A, Forsberg RA (1995a) Discovery of 2n gametes in tetraploid oat *Avena vaviloviana*. Euphytica 81:1-6
Katsiotis A, Forsberg RA (1995b) Pollen grain size in four ploidy levels of genus *Avena*. Euphytica 83:103-108

Kihara H (1982) Wheat studies – retrospect and prospects. Developments in crop science 3. Kodansha Ltd., Tokyo

Kłyk B (2005) Zmienność mikrostrukturalna niektórych gatunków rodzaju *Brachypodium* P. Beauv. Dissertation, University of Wrocław, Wrocław

Kruskal JB (1964) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika 29:1–27

Kosina R (2007) Some topics of the grass mosaics. In: Frey L. (ed) Biological issues in grasses. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, pp 159-167

Kosina R (2015) Variation of reproduction in some species of the tribe Aveneae (Poaceae). Modern Phytomorphology 8: 11-14

Kosina R, Florek M (2011) On some characteristics of *Avena* (Poaceae) pollen grains. In: Frey L. (ed) Advances in grass biosystematics. W. Szafer Institute of Botany, Polish Academy of Science, Kraków, pp 107-117

Kosina R, Florek M, Tomaszewska P (2014) Pollen grain morphogenesis in Triticeae and *Avena* amphiploids. Ann Wheat Newslet 60:102–103

Kosina R, Tomaszewska P (2015) Variability of breeding system, caryopsis microstructure and germination in annual and perennial species of the genus *Brachypodium* P. Beauv. Genetic Resources and Crop Evolution 63:1003-1021
Kushwaha N, Zadoo SN, Choubey RN (2004) Breakdown of polyploidy and isolation of stable polysomics in amphidecaploid Avena sativa L. × A. maroccana Gdgr. Cytologia 69:41-47

Leighty CE, Sando WJ (1924) Pistillody in wheat flowers. J Hered 15:263-268 https://doi.org/10.1093/oxfordjournals.jhered.a102468

Li X-F, Liu S-B, Gao J-R, Lu W-H, Wang H-G (2005) Abnormal pollen development of bread wheat-Leymus mollis partial amphiploid. Euphytica 144:247-253

Linde-Laursen I (1993) Cytogenetic analysis of Miscanthus ‘Giganteus’, an interspecific hybrid. Hereditas 119:297-300

Linde-Laursen I, Bothmer R von (1993) Aberrant meiotic divisions in a Hordeum lechleri × H. vulgare hybrid. Hereditas 118:145-153

Liu Q, Zhao N-X, Hao G (2004) Pollen morphology of the Chloridoideae (Gramineae). Grana 43:238-248

Loskutov IG (2001) Interspecific crosses in the genus Avena L. Russ J Genet 37:467–475

Ma GH, Huang XL (2007) Cytological and embryological studies on apospory in Bothriochloa ischaemum L. Acta Biol Hung 58: 421-429

Malallah GA, Attia TA (2003) Cytomixis and its possible evolutionary role in a Kuwaiti population of Diplotaxis harra (Brassicaceae). Bot J Linn Soc 143:169-175

Malecka A (2008) Mejoza u Allium senescens subsp. montanum. Dissertation, University of Wrocław, Wrocław

Mendes-Bonato AB, Junqueira Filho RG, Pagliarini MS, do Valle CB, de Oliveira Penteado MI (2002) Unusual cytological patterns of microsporogenesis in Brachiaria decumbens:
abnormalities in spindle and defective cytokinesis causing precocious cellularization.

Cell Biol Int 26:641-646

Meo AA (1999) Impact of pollen and intergenetic crosses between graminaceous (Poaceae) plants. Pak J Biol Sci 2:809-812

Mujeeb KA, Thomas JB, Rodriguez RR, Waters RF, Bates LS (1978) Chromosome instability in hybrids of *Hordeum vulgare* L. with *Triticum turgidum* and *T. aestivum*. J Hered 69:179–182 https://doi.org/10.1093/oxfordjournals.jhered.a108920

Murai K, Tsunewaki K (1993) Photoperiod-sensitive cytoplasmic male sterility in wheat with *Aegilops crassa* cytoplasm. Euphytica 67:41-48

Ottaviano E, Mulcahy DL (1989) Genetics of Angiosperm pollen. AdvGenet 26:1-64

Paczos-Grzędka E (2003) Badania cytogenetyczne i molekularne mieszańców międzygatunkowych heksaploidalnego owsa *Avena sativa* L. × *Avena sterilis* L. oraz form wyjściowych. Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin 229:21-32

Platje J (2003) Charakterystyka cytogenetyczna niektórych taksonów rodzaju *Magnolia* L. Dissertation. University of Wrocław, Wrocław

Protasevich RT (1984) Morfologo-anatomické osobennosti rastenij s mužskoj steril’nost’ju. Nauka i Technika, Minsk

Quarin CL, Espinoza F, Martinez EJ, Pessino SC, Bovo OA (2001) A rise of ploidy level induces the expression of apomixis in *Paspalum notatum*. Sex Plant Reprod 13:243–249 https://doi.org/10.1007/s004970100070

Ressayre A, Godelle B, Mignot A, Gouyon PH (1998) A morphogenetic model for pollen aperture pattern in flowering plants. J Theor Biol 193:321-334

Richards AJ (1986) Plant breeding systems. George Allen & Unwin, London
Rohlf FJ (1981) Spatial representation of phylogenetic trees computed from dissimilarity matrices. Int. Symp. on Concept and Method in Paleontology, Barcelona:303–311

Rohlf FJ (1994) NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 1.80. Exeter Software, New York

Sakata T, Takahashi H, Nishiyama I, Higashitani A (2000) Effects of high temperature on the development of pollen mother cells and microspores in barley *Hordeum vulgare* L. J Plant Res 113:395-402

Sheidai M, Koobaz P, Zehzad B (2003) Meiotic studies of some *Avena* species and populations in Iran. J Sci, Islam Rep Iran 14(2): 121-131

Southworth D, Pfahler P (1992) The effects of genotype and ploidy level on pollen surface sculpturing in maize (*Zea mays* L.). Amer J Bot 79: 1418-1422

Staszak A (2004) Morfometria *Prunus spinosa* L. z populacji okolic Wrocławia. Dissertation, University of Wrocław, Wrocław

Stuczyński M, Stuczyńska J, Stuczyńska E (1994) Niezredukowane gamety w hodowli traw. Genet Pol 35A:3-9

Teng N, Huang Z, Mu X, Jin B, Hu Y, Lin J (2005) Microsporogenesis and pollen development in *Leymus chinensis* with emphasis on dynamic changes in callose deposition. Flora 200:256-263

Thomas H, Peregrine WTH (1964) Chromosome mosaics in synthetic amphiploids in the *Avenae*. Chromosoma 15:123-131

Tomaszewska P, Kosina R (2018) Instability of endosperm development in amphiploids and their parental species in the genus *Avena* L. Plant Cell Rep 37: 1-14
Tomaszewska P, Kosina R (2021) Cytogenetic events in the endosperm of amphiploid *Avena magna* x *A. longiglumis*. J Plant Res. https://doi.org/10.1007/s10265-021-01314-3

Tomaszewska P, Pellny TK, Hernández LM, Mitchell RAC, Castiblanco V, De Vega JJ, Schwarzacher T, Heslop-Harrison JS (2021a) Flow cytometry-based determination of ploidy from dried leaf specimens in genomically complex collections of the tropical forage grass *Urochloa* s.l. Genes 12: 957

Tomaszewska P, Vorontsova M, Renvoize SA, Ficinski SZ, Tohme J, Schwarzacher T, Castiblanco V, De Vega JJ, Mitchell RAC, Heslop-Harrison JS (2021b) Complex polyploid and hybrid species in an apomictic and sexual tropical forage grass group: genomic composition and evolution in *Urochloa* (*Brachiaria*) species. https://doi.org/10.1101/2021.02.19.431966

Warzych A (2001) Zmienność mikrostrukturalna *Avena fatua* L. i taksonów pokrewnych. Dissertation, University of Wrocław, Wroclaw

Wojciechowska B, Pudelska H (2002) Hybrids and amphiploids of *Aegilops ovata* L. with *Secale cereale* L.: production, morphology and fertility. J Appl Genet 43:415-421

Wojciechowska B, Pudelska H (2005) Production and characterization of amphiploids of *Aegilops kotschyi* and *Ae. biuncialis* with *Secale cereale*, and of backcross hybrids of *Ae. biuncialis* × *S. cereale* amphiploids with 2x and 4x *S. cereale*. J Appl Genet 46: 157-161

Zhao H, Bughrara SS, Wang Y (2007) Cytology and pollen grain fertility in creeping bentgrass interspecific and intergeneric hybrids. Euphytica 156:227-235
## Tables

**Table 1** List of studied accessions of oat amphiploids and their parental species

| Amphiploid and parental species | Abbreviation | Accession number | Origin | Ploidy level |
|---------------------------------|--------------|------------------|--------|--------------|
| A. barbata × A. sativa subsp. nuda | b/sn | CIav7903** | - | octoploid |
| A. eriantha × A. sativa | e/sa | PI458781** | - | octoploid |
| A. barbata × A. sativa | b/sa | CIav7901** | - | hexaploid |
| A. fatua × A. sterilis | f/ste | Clav9367** | - | hexaploid |
| A. magna × A. longiglumis | m/l | Clav9364** | - | hexaploid |
| A. abyssinica × A. strigosa | a/str | Clav7423** | - | tetraploid |
| A. fatua | Af | RK | Poland | hexaploid |
| A. sterilis | Aste | PI311689** | Israel | hexaploid |
| A. sativa | Asa | RK | Poland | hexaploid |
| A. barbata | Ab | AVE1938* | Spain | tetraploid |
| A. abyssinica | Aa | PI331373** | Ethiopia | tetraploid |
| A. magna | Am | 1786*** | Morocco | tetraploid |
| A. strigosa | Astr | 51624* | Belgium | diploid |
| A. longiglumis | Al | PI367389** | Portugal | diploid |
| A. eriantha | Ae | Clav9051** | England | diploid |

* Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Braunschweig, Germany; ** National Small Grains Collection, Aberdeen, Idaho, USA; *** Vavilov Institute of Plant Industry, St. Petersburg, Russia; RK – collected by R. Kosina

**Table 2** Viability and size of pollen grains of oat amphiploids and their parental species

| Amphiploid and parental species | Viability of pollen grains [%] | Length of pollen grains [µm] |
|---------------------------------|---------------------------------|-------------------------------|
|                                 | Year 1 | Year 2 | Year 3 | Mean | Year 1 | Year 2 | Year 3 | Mean |
| A. barbata × A. sativa subsp. nuda | - | 90.5 | 91.2 | 91.1 | - | 48.8 | 48.1 | 48.3 |
| A. eriantha × A. sativa | 84.4 | 98.3 | 97.6 | 90.5 | 53.9 | 50.5 | 56.0 | 53.9 |
| A. barbata × A. sativa | 92.8 | 96.8 | 96.7 | 96.0 | 42.0 | 41.5 | 46.8 | 43.6 |
| A. fatua × A. sterilis | 82.7 | 98.0 | 98.0 | 95.5 | 43.3 | 49.5 | 50.4 | 48.3 |
| A. magna × A. longiglumis | 96.6 | 95.6 | 91.1 | 95.1 | 51.8 | 46.6 | 50.5 | 49.8 |
| A. abyssinica × A. strigosa | 96.5 | 88.3 | 98.6 | 94.2 | 44.3 | 36.9 | 43.7 | 43.4 |
| A. fatua | 98.4 | 98.7 | 99.4 | 98.9 | 45.4 | 44.9 | 49.1 | 46.7 |
| A. sterilis | 96.9 | 99.5 | 98.8 | 98.5 | 50.8 | 50.1 | 50.2 | 50.4 |
| A. sativa | 99.0 | 88.5 | 98.2 | 96.5 | 43.0 | 46.5 | 40.9 | 43.0 |
| A. barbata | 97.6 | - | 99.0 | 98.1 | 44.5 | - | 43.7 | 44.0 |
| A. abyssinica | 96.3 | - | 97.7 | 97.4 | 45.3 | - | 43.5 | 44.0 |
| A. magna | 98.1 | 97.7 | 98.6 | 98.2 | 45.2 | 41.9 | 49.5 | 45.8 |
| A. strigosa | 96.6 | 99.1 | 98.9 | 98.3 | 44.1 | 42.7 | 45.2 | 44.0 |
| A. longiglumis | - | 98.1 | 95.5 | 96.3 | - | 42.9 | 44.5 | 44.3 |
| A. eriantha | 98.1 | - | 97.5 | 97.6 | 41.0 | - | 36.9 | 37.6 |
Table 3 Pearson’s correlation coefficients matrix of the two pollen grain characteristics, viability and length, in the three growing seasons

|     | V1     | V2     | V3     | L1     | L2     |
|-----|--------|--------|--------|--------|--------|
| V2  | -0.24  |        |        |        |        |
| V3  | -0.01  | 0.25   |        |        |        |
| L1  | -0.26  | 0.31   | -0.32  |        |        |
| L2  | -0.46  | 0.27   | -0.28  | 0.60*  |        |
| L3  | -0.58* | 0.57*  | -0.11  | 0.73***| 0.55*  |

V1, V2, V3 - viability of pollen grains in the three growing seasons; L1, L2, L3 - length of pollen grains in the three growing seasons; *, ***, - significance levels at α = 0.05, 0.001, respectively

Table 4 Frequency of anomalous pollen grains (three-year average)

| Amphiploids and parental species                  | Anomalous pollen grains [%] | micropollens | unreduced | irregular |
|---------------------------------------------------|-----------------------------|--------------|-----------|-----------|
| A. barbata × A. sativa subsp. nuda                |                             | 0.89         | 0         | 0.05      |
| A. eriantha × A. sativa                          |                             | 0.62         | 0.02      | 0.06      |
| A. barbata × A. sativa                           |                             | 0.28         | 0         | 0.01      |
| A. fatua × A. sterilo                            |                             | 0.90         | 0         | 0.01      |
| A. magna × A. longiglimis                        |                             | 0.12         | 0.01      | 0.02      |
| A. abyssinica × A. strigosa                      |                             | 0.22         | 0.02      | 0.01      |
| A. fatua                                         |                             | 0.18         | 0         | 0.01      |
| A. sterilis                                      |                             | 0.30         | 0         | 0         |
| A. sativa                                        |                             | 0.19         | 0         | 0         |
| A. barbata                                       |                             | 0.03         | 0         | 0.02      |
| A. abyssinica                                    |                             | 0.46         | 0         | 0.03      |
| A. magna                                         |                             | 0.17         | 0         | 0.002     |
| A. strigosa                                      |                             | 0.24         | 0.01      | 0         |
| A. longiglimis                                   |                             | 0.09         | 0         | 0         |
| A. eriantha                                      |                             | 0.23         | 0         | 0         |
**Table 5** Frequency of pollen grains with a certain number of poruses (%)

| Amphiploids and parental species | 1 porus | 2 poruses | 3 poruses | 4 poruses | > 4 poruses |
|----------------------------------|---------|-----------|-----------|-----------|------------|
| A. barbata × A. sativa subsp. nuda | 97.1    | 2.28      | 0.54      | 0.13      | 0          |
| A. eriantha × A. sativa          | 99.4    | 0.43      | 0.11      | 0         | 0.05       |
| A. barbata × A. sativa           | 99.9    | 0.11      | 0         | 0         | 0          |
| A. fatua × A. sterilis            | 99.4    | 0.62      | 0         | 0         | 0          |
| A. magna × A. longiglumis         | 99.8    | 0.22      | 0         | 0         | 0          |
| A. abyssinica × A. strigosa       | 99.8    | 0.21      | 0         | 0         | 0          |
| A. fatua                         | 100     | 0         | 0         | 0         | 0          |
| A. sterilis                       | 100     | 0         | 0         | 0         | 0          |
| A. sativa                         | 100     | 0         | 0         | 0         | 0          |
| A. barbata                        | 100     | 0         | 0         | 0         | 0          |
| A. abyssinica                     | 100     | 0         | 0         | 0         | 0          |
| A. magna                          | 100     | 0         | 0         | 0         | 0          |
| A. strigosa                       | 100     | 0         | 0         | 0         | 0          |
| A. longiglumis                    | 100     | 0         | 0         | 0         | 0          |
| A. eriantha                       | 100     | 0         | 0         | 0         | 0          |

**Tabla 6** Pearson’s correlation coefficient matrix for the ten characteristics of pollen grains (three growing seasons)

|          | V     | L     | MP    | UR    | IR    | 1P    | 2P    | 3P    | 4P    |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| L        | -0.51*|       |       |       |       |       |       |       |       |
| MP       | -0.66**| 0.42  |       |       |       |       |       |       |       |
| UR       | -0.52*| 0.33  | 0.03  |       |       |       |       |       |       |
| IR       | -0.78***| 0.58*| 0.59*| 0.37  |       |       |       |       |       |
| 1P       | 0.73***| -0.31 | 0.73***| 0.01 | -0.64**|       |       |       |       |
| 2P       | -0.73***| 0.32 | 0.75***| -0.03| 0.62**| -1.00***|       |       |       |
| 3P       | -0.67**| 0.28 | 0.63**| 0.03 | 0.65**| -0.98***| 0.96***|       |       |
| 4P       | -0.56*| 0.16 | 0.57*| -0.15| 0.52*| -0.96***| 0.95***| 0.98***|       |
| >4P      | -0.56*| 0.59*| 0.30 | 0.60*| 0.67**| -0.10  | 0.08  | 0.13  | -0.07 |

V – viability of pollen grains, L – length of pollen grains, MP - micropollens, UR – unreduced pollen grains, IR – irregular pollen grains, 1P – pollens with one porus, 2P – pollens with two poruses, 3P – pollens with three poruses, 4P – pollens with four poruses, > 4P – pollens with more than four poruses.
Fig. 1 Minimum spanning tree (MST) of amphiploids and parental species (OTUs) of the genus *Avena* in an ordination space (x, y, and z-axes) and created by application of Kruskal’s non-metric multidimensional scaling method (Rohlf 1994). OTUs were described by pollen viability and pollen length in the three growing seasons. Abbreviations in the Table 1. The distances between pairs of OTUs in the minimum spanning tree (MST) are as follows (extreme distances are bolded): b/sn - m/l 0.869; m/l - Aste 0.775; Aste - Am 0.703; Am - Af 0.245; Af - Astr 0.398; Astr - Aa 0.469; Aa - Ab 0.205; Astr - Al 0.489; Al - b/sa 0.296; Aa - a/str 0.602; a/str - Ae 0.664; Ae - Asa 0.743; Al - f/st 1.040; f/st - e/sa 1.231
**Fig. 2** A diagram of the linear regression showing relationship between pollen grain length and ploidy level of oat amphiploids and parental species. Length of pollen grains in µm

\[ y = 37.75 + 1.59x \]
Fig. 3 Anomalous morphotypes of pollen grains. a – *A. fatua* × *A. sterilis*; b,c – *A. eriantha* × *A. sativa*; d – *A. abyssinica* × *A. strigosa*; e – *A. eriantha* × *A. sativa*; f,g – *A. eriantha* × *A. sativa*; h – *A. abyssinica* × *A. strigosa*; i – *A. barbata* × *A. sativa*; j – *A. fatua* × *A. sterilis*; k – *A. eriantha* × *A. sativa*. Acetocarmine staining, black arrows indicate poruses. Scale bars = 20μm
Fig. 4 Different morphotypes, number and position of poruses in pollen of oat amphiploids. a - A. magna × A. longiglumis, pollen with one porus; b,c - A. eriantha × A. sativa, pollens with two poruses; d - A. magna × A. longiglumis, pollen with two poruses; e - A. magna × A. longiglumis, pollen with two poruses; f - A. barbata × A. sativa subsp. nuda, pollen with three poruses. Acridine orange fluorescence. White arrows indicate poruses. Scale bars = 20μm
Fig. 5 Minimum spanning tree (MST) of amphiploids and parental species (OTUs) of the genus *Avena* in an ordination space (x-, y-, and z-axes) and created by application of Kruskal’s non-metric multidimensional scaling method (Rohlf 1994). OTUs were described by ten traits of pollen (viability, length, micropollens, unreduced pollen, irregular pollen, pollen with 1 porus, pollen with 2 poruses, pollen with 3 poruses, pollen with 4 poruses, pollen with more than 4 poruses). Abbreviations in the Table 1. Some OTUs are hidden, Ab behind Astr and Al behind b/sa. The distances between pairs of OTUs in the minimum spanning tree (MST) are as follows (extreme distances are bolded): b/sn - f/ste 1.088; f/ste - m/l 0.460; m/l - Aste 0.639; Aste - Af 0.722; Af - Am 0.331; Am - Ab 0.463; Ab - Astr 0.002; Astr - Aa 0.231; Aa - Al 0.233; Al - b/sa 0.096, Aa - Asa 0.236; b/sa - a/str 0.505; Asa - Ae 1.112; m/l - e/sa 1.265