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TEST FUSION IN ADULT FORAMINIFERA: A REVIEW WITH NEW OBSERVATIONS OF AN EARLY EOCENE *NUMMULITES* SPECIMEN

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Abstract

In foraminifera, so-called “double tests” usually arise due to abnormal growth originating mainly from twinning, but may also be caused by irregularities in the early chambers and by regeneration after test injury that modifies the direction of growth. A fourth cause of double tests has only rarely been reported: the fusion of the tests of two adult individuals. We studied an early Eocene *Nummulites* double test consisting of two adult individuals that fused after an extended period of independent growth. The specimen was studied using computed tomography with micrometric resolution (micro-CT) that allowed bi- and three-dimensional visualization of the internal structure. Before fusion each individual test had 30–36 chambers, which, by comparison with growth rates in recent nummulitids, implies at least three months of independent growth. After fusion, the compound test grew in two spirals that fused after about one whorl and then continued in a single spiral.

To fuse their tests, either adult individuals have to be forced to do so or the allorecognition (ability to distinguish between self and another individual) mechanisms must fail. A possible explanation for the merged *Nummulites* tests in this study is forced fusion in attached individuals after surviving ingestion and digestion by a metazoan. Alternatively, environmental stress could lead to a failure of allorecognition mechanisms and/or foraminiferal motility. Once fused, subsequent growth seems to be determined mainly by the relative orientation of individual tests. In any case, the frequency in which adult fusion occurs remains unknown.

INTRODUCTION

Abnormal growth in foraminifera can lead to a wide range of test malformations (e.g., Polovodova & Schönfeld, 2008). So-called double tests in which the test grows in two directions are a particular type of abnormality. Double tests have been reported since the middle of the 19th century (e.g., Schultze, 1854; Carpenter, 1856; Rhumbler, 1902). Rhumbler (1911) distinguished between “true double shells,” those having two proloculi,

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and “cleavage monsters,” those with a single proloculus. Double tests in Eocene *Nummulites* have been reported by several authors (e.g., de la Harpe, 1881; Regè, 1916; Marchesini & Facca, 1940; Abrard, 1945, 1951; Spiegler, 1958; Kecskeméti, 1962a, b; De Zanche, 1966; Pavlovec, 1976).

Double tests are usually related to twinning, i.e., fusion or incomplete separation of megalospheric embryos during schizogony. Tests having two or more embryonic apparatuses are called polyvalent individuals (Le Calvez, 1934, 1938; Hottinger, 2006), irrespective of whether the test is normal or irregular.

Juveniles released from the mother test in schizogony usually have two or three chambers. The juveniles may become fused at their proloculi or at these other chambers immediately after schizogony. After test fusion individuals grow either a normal test or a test with a double structure, such as double spiral or supplementary discs. The fusion of juveniles has been related to crowding in the brood chamber of the mother test or in a reproductive cyst before being released (Cole, 1960; Arnold, 1964; Thompson, 1964; Meriç et al., 2001). Examples of such crowding can be seen in *Sorites orbiculus* (Forskål) (Kloos, 1984, fig. 1), *Amphisorus hemprichii* Ehrenberg (Hottinger, 2006, fig. 24A), and *Neorotalia* sp. (Hottinger, 2006, fig. 24F). Krüger et al. (1997) reported the “coalescence of two or several individuals,” thus forming “Siamese twins” in cultured *Cyclocypeus carpenteri* Brady, in which up to 2540 offspring from the same mother test were observed. The delayed release of juveniles has been attributed to excessive wall thickness of the cyst or reproductive cyst under certain ecological conditions (Cole, 1960) or to rapid algal overgrowth of the reproductive chamber, preventing or retarding the young from escaping (Arnold, 1964). Hypersalinity might inhibit or slow down the movements of the young, making their dispersal difficult after being released by the schizont (Stouff et al., 1999).

Recently Langer et al. (2009) have shown test deformities in 27% of the megalospheric juveniles of *Peneroplis* sp., which are densely packed within the limited space of a brood chamber. The deformations consisted of indentations on the test surface resembling imprints of neighboring tests, implying asynchronous calcification. This example shows that, when calcification of the megalospheric juveniles takes place within the parental test, crowding of juveniles does not necessarily imply test fusion and thus twinning, at least in porcellaneous species.

Twinning also occurs in species whose reproductive cycle does not take place in the mother test or in a reproductive cyst, such as in *Ammonia* (Stouff et al., 1999). According to Krüger et al. (1997), *C. carpenteri* juveniles can also fuse after being released from the parental test, but only during the first few days after their release, when hundreds of individuals are crowded near the parental test.

Twinning is not rare in the fossil record, with individuals with two or more embryos having been reported in most groups of larger foraminifera, including Eocene *Nummulites* (e.g., Popescu-Voitesti, 1908; Regè, 1916). In some cases the number of merged individuals is high: up to six embryos in *Planorbulina mediterraneensis* (Le Calvez, 1938), five in “*Orbitolites complanata*” [= *A. hemprichii*] (Heron-Allen, 1915), seven in *Pseudophragmina*

(*Proporocyclina*) *zaragosensis* (Vaughan) (Cole, 1963, pl. 7, fig. 4), and nine in *Alveolina canavarii* Checchia-Rispoli (Checchia-Rispoli, 1905). As pointed out by Le Calvez (1938), the embryos in such polyvalent individuals are similar in size. Twinning in microspheric forms has occasionally been reported, but not necessarily in plastogamic species in which microspheric juveniles may be crowded in the brood chamber (e.g., *P. mediterraneensis*, Le Calvez, 1938).

Double tests can also occasionally originate from anomalies in the early development of a single juvenile, e.g., the building of two second or third chambers, from which separate whorls or discs originate, as in *Ammonia tepida* (Cushman) and *Elphidium crispum* (Linnaeus) (Stouff et al., 1999). However, individuals of *Cyclocypeus* and *Heterostegina* with two deuterocoelons develop into normal adults (Röttger & Spindler, 1976; Krüger et al., 1997).

Although twin individuals are produced by fusion between individuals of the same size (i.e., the same age, Le Calvez, 1938), the attachment and subsequent growth of a juvenile on the parental test has also been reported in *Ammonia tepida* from laboratory cultures (Stouff et al., 1999) and in *Amphistegina gibbosa* d'Orbigny from stressed populations (Hallock, 2000). Popescu-Voitești (1908) interpreted an abnormal test of "*Nummulites* (*Hantkenia*) *Tchihatcheffi* (sic)" d'Archiac & Haime (= probably an A form of *N. maximus* d'Archiac according to Schaub, 1981) in this way. The sections of the specimen (Popescu-Voitești, 1908, figs. 4–6) are not centered and the abnormal shape could be due to other causes.

Wilde (1965) described two examples of "engulfment" of juveniles by adult individuals, either congeneric (or conspecific), as in *Pseudoschwagerina* (Wilde, 1965, pl. 20, fig. 3), or between genera, with a *Schubertella* juvenile engulfed by a mature *Triticites* (Wilde, 1965, pl. 20, fig. 2). As stated by Wilde, in neither case is it clear whether the juvenile was alive or whether it continued to live and grow.

Apparent double tests can result from plastogamy, in which two gamonts attach to each other at the apertural face to exchange their gametes in the shared common space. The mother individuals then die, and the paired tests can remain together. These paired tests can fossilize either before or after reproduction. Because juveniles originating via plastogamy grow in a crowded and limited space (e.g., see fig. 3 in Preobrazhenskaya & Tarasova, 2004), plastogamy could lead to the fusion of microspheric juveniles. Plastogamic reproduction has been postulated in some Eocene *Nummulites*, based on the occurrence of some paired tests (Mukhopadhyay, 2003, 2007). The evidence for this is not convincing however, and it is likely that these *Nummulites* tests became paired due to random association in the sediment and compaction during lithification.

Other "double tests" found in the literature are actually falsely identified. Reported cases of test "penetration" in different genera (e.g., "*Orbitoides*" with *Assilina* and *Nummulites*; de la Harpe, 1881; Popescu-Voitești, 1908) are due to diagenetic compaction. Some tests showing an unusual change in their generic growth plan have been interpreted as interspecific and intergeneric hybrids (e.g., Heron-Allen, 1915; Meriç et al., 2008, 2012). A review of the diagnostic characters of these taxa should be taken into account before reaching such an

improbable conclusion. An extremely rare cause of double tests, which is the object of this study, is the test fusion of two adult individuals. Very few examples of such fusion are found in the literature. Wilde (1965) illustrated a specimen of clearly fused adult individuals of *Schwagerina* sp. (Wilde, 1965, pl. 19, fig. 5), together with other questionable double tests in axial sections that could have been caused by abnormal growth following fracture (Wilde, 1965, pl. 20, figs. 1, 4, 5). Bradley (1956) and Kahler (1988, figs. 23, 26) reported intergrowth of adult fusulinid tests from uncentered sections, and thus are questionable.

A good example of apparent fusion of independently grown individuals is found in a double test of *Nummulites millecaput* Boubée, produced by abnormal growth after injury and fracture of the test (Kecskeméti, 1962a, b). Figure 4 in Kecskeméti (1962a; reproduced in Kecskeméti, 1962b, fig. 1.4) is a drawing in which the two parts of the double test are cut in an uncentered axial section. A common whorl appears to envelope two individuals, which exhibit a minimum of 10 and six whorls of apparently independent growth. This example illustrates the difficulty of studying abnormally shaped tests, particularly in thin sections. A specific case is found in the Y-shaped *Parafusulina* specimen illustrated by Bradley [1956, pl. 40 (not 43 as written in the text), fig. c], which appears to be a fragmented test attached perpendicularly to another complete test that subsequently grew together. Although Bradley interpreted this as being “due to fracture and regeneration at the fractured end,” the number of whorls of the two parts inside the enveloping whorls point to the possible fusion of adult tests. The section does not allow for further explanations.

The study of teratological tests is difficult because their outer morphology does not reflect the morphology of the abnormally shaped interior, and sections are needed. Double tests are usually interpreted to be conjoined twins, although evidence of two or more embryos in a section is usually missing. Thin sections, however, are bidimensional, and abnormal tests with double direction of growth require study in three dimensions. The only way to study the shape of such tests and to investigate their ontogeny is to use computed tomography with micrometric resolution (microCT).

The use of microCT, although only successful in those specimens possessing high density contrast between test structure and chamber infilling (e.g., empty tests; Briguglio et al., 2011), prevents the destruction of the specimen, and allows three-dimensional study by producing virtual sections of the scanned object in any direction. These can be used to better visualize complicated morphologies and to show them as virtual models (Briguglio & Benedetti, 2012; Briguglio et al., 2014).

Here we report an example of test fusion of two adult Eocene *Nummulites* investigated by means of microCT. This methodology produced precise oriented sections of the abnormal test and the reconstruction of the separate growth of two individuals prior to fusion, followed by subsequent common growth into a single test.

MATERIALS AND METHODS

The fused individuals reported herein come from level IV (Douvillé & O’Gorman, 1929) of the Tuilerie de Biron (Marnes de Biron, Gave du Pau region, southern France; see

Ferràndez-Cañadell, 1997 for site details), collected by the senior author in 1987. These beds correspond to Shallow Benthic Zone SBZ 14 of Serra-Kiel et al. (1998).

The specimen, identified as *Nummulites aspermontis* Schaub, was scanned using a Skyscan 1163 high-energy microCT at the Department of Paleontology at the University of Vienna, Austria. The main parameters used for the scan are reported in Table 1. Micrographs were taken by an FEI Quanta 200 Scanning Electronic Microscope located at the Serveis Científic-Tècnics of the University of Barcelona.

RESULTS

The specimen shows a double test with two merging lenticular parts (Fig. 1). The microCT scan revealed that it corresponds to two individuals (from here on called A and B) with distinct proloculi and separate juvenile stages. The two individuals are conspecific, with a similar proloculus diameter (407×502 and 426×480 μm ; Figs. 1.2a, b). The distance between the centers of the proloculi is 1037 μm , whereas the distance between the proloculi walls is 555 μm . The angle between the two initial planispiral tests A and B is 81° (Fig. 1.2a). Their initial growth differed slightly: the first and second whorls have 7 and 23 chambers in individual A, and 8 and 25 chambers in individual B (Fig. 2).

The two tests contact at the 31st chamber of A and the 18th of B (Figs. 2, 3.1), although these were not necessarily the youngest chambers at the time of fusion. Subsequently each individual continued building separate involute spirals, covering both tests. After one whorl, the spiral of specimen B split: five chambers grew following the underlying test of specimen A and the other chambers continued growing in their own spiral (Fig. 3.2) up to chamber 60. Specimen A continued building its own spiral up to chamber 54. At chambers 54 and 60, the spirals converged and these chambers came into frontal contact (Fig. 3.3), resulting in the growth of a single spiral. The next chamber is common and corresponds to the 55th chamber of A and/or the 61st of B, thus setting the ontogenetic difference between individuals in six chambers, and indicating that when the two individuals merged their tests, the individuals had 36 (A) and 30 (B) chambers (Fig. 2).

The common spiral envelops both former spirals and has at least 63 more chambers, thus indicating favorable life conditions. After chamber 94A/99B it splits again, with at least 11 chambers in one direction and 7 in another. The specimen is broken in its last whorl and the chambers are not preserved, but it built at least one more whorl, with 30–40 additional chambers.

In summary, two conspecific adult individuals fused their tests when they had 30 (A) and 36 (B) chambers, then continued growing with two spirals at 81° for about one whorl until the spirals fused, and then built a common spiral.

DISCUSSION

Studied Specimens Are True Fused Adult Individuals

Double tests are usually caused by the early fusion of juveniles within the reproductive cyst or the mother test during schizogony, and rarely (only observed in laboratory culture) by the

fusion of a juvenile with the mother test (Stouff et al., 1999; Hallock, 2000) or the fusion of two microspheric tests (Le Calvez, 1938). Abnormal duplication of the second or third chamber can also give rise to tests with double growth (Krüger et al., 1997; Stouff et al., 1999). The double test of *Nummulites* reported herein does not correspond to any of these cases; it clearly originated via the fusion of two normal adult tests after a period of independent life. The fused individuals have ~30 and 36 chambers of separate, independent growth. According to the growth rates reported in the Recent nummulitids *Palaeonummulites venosus* (Hohenegger & Briguglio, 2014) and *Heterostegina depressa* (Röttger & Spindler, 1976), it would have taken ~3 months to build these tests. During schizogony in *C. carpenteri* the remaining cytoplasm of the mother test is used to spread the offspring (Krüger et al., 1997). After this initial dispersion, the young could disperse considerably by their own movement and by displacement due to water flow within three months.

Test Fusion in Fusulinids

Wilde (1965) presented several examples of fusulinid double tests in thin section. Some of his interpretations of these specimens as polyvalent or fused individuals are questionable, because the sections do not show the initial chambers of two specimens and the morphology could actually be due to abnormal growth after injury (Wilde, 1965, pl. 20, fig. 1: *Sumatrina longissima* Deprat; fig. 4: *Parafusulina* sp.; and fig. 5: *Fusulina* sp.), similar to the specimen of *Parafusulina* sp. in Bradley (1956, pl. 40, fig. c), or to twinning, as in the specimen of *Quasifusulina longissima* (von Möller) in Kobayashi & Altiner (2008, pl. 8, fig. 4). One of Wilde's specimens (pl. 19, fig. 5), however, clearly shows the fused tests of two adult *Schwagerina* sp., one of which had built ~41 chambers and the other >60 chambers independently (Fig. 4). The two tests seem to be attached at the broken chambers 33–35 of one specimen and 55–56 of the other. Following fusion, the individuals continued to grow, enveloping each other's test. However, the smaller individual seems to have built only two more chambers, after which growth was dominated by the larger specimen (Fig. 4). In this case, the two individuals differ in at least 16, but possibly up to 26 chambers, and thus might not be siblings from the same asexual brood. Together with the specimen of *Nummulites* reported herein, this specimen of *Schwagerina* seems to be the only reported clear example of test fusion among adult individuals.

Allorecognition and Natural Repulsion Between Individuals

Allorecognition (i.e., the capacity of an organism to distinguish its own tissues from those of another) has been recognized in foraminifera for some time. In 1895, Jensen noted that two individuals of the same species, "*Orbitolites complanata*" (= *A. hemprichii*) and *Amphistegina gibbosa* d'Orbigny, could not be forced to fuse. Their cytoplasm did not blend at contact, but on the contrary appeared to repel that of the other. The exception was when they were very young. Jensen (1895, p. 195) reported that the day after they were released from the mother test, when measuring about 0.2 mm in size, juveniles of *O. complanata* (= *A. hemprichii*) extended their pseudopodia towards each other and fused them. However, when older individuals were brought together with the younger individuals, the pseudopodia contracted and never fused. Although Jensen did not repeat the experiment with offspring from different mothers, or check when individuals started to repel each other, it can be

reasonably concluded that fusion between individuals typically occurs only during the very initial ontogenetic stages.

Allorecognition plays an important role in colonial marine invertebrates such as sponges, ascidians, cnidarians, and bryozoans (Elgar & Crozier, 1989). For example, when two bryozoan colonies meet, they might fuse into a single colony if their genotype is compatible, but if they differ genetically, they may respond aggressively by fighting for space or attacking each other (Hughes et al., 2004).

A similar behavior may occur in Foraminifera. Two different individuals may fuse if they are young siblings from the same asexual brood, but adult individuals will repel each other. This can be observed in sessile attached species, where growth at the margins stops when two individuals contact (e.g., in *Planorbulina*, see figure in table 5 in Richardson-White & Walker, 2011). However, adult individuals might become fused if they are forced, as shown experimentally by Schwab & Schwab-Stey (1980). These authors forced the fusion of up to 12 individuals of *Myxotheca arenilega* Schaudinn into giant cells by carefully removing the cell bodies from their organic tests. Although not stated in their paper, the fused individuals were probably siblings because the individuals came from longterm laboratory cultures.

A fused test does not imply completely fused cells, however. Even in twinning fusion individuals remain relatively independent, thus producing abnormal growth in two directions. As already pointed out by Heron-Allen (1915), in plastogamy the cytoplasm of the two individuals fuses, but there is no fusion of the nuclei (karyogamy). Le Calvez (1938, p. 242) similarly observed that in a twinned specimen of *Planorbulina mediterraneensis* the cytoplasm, but not the nuclei, was undifferentiated. Furthermore, in this specimen the chambers formed after fusion were approximately twice the volume of the chambers of a normal individual with a proloculus of similar size, pointing to the combined activities of each cytoplasm and nucleus, which he referred to as “true parabiosis.” Wilde (1965) stated that fused tests do not prove protoplasmic fusion that cannot be demonstrated adequately even in twins fused at a very early stage of growth. He illustrated this point with an example of a *Fusulina* twin specimen (Wilde, 1965, pl. 18, figs. 5, 6), in which there are double tunnels for a number of whorls, concluding that “each individual acted as a unit for some time, even though fused with the other.”

In summary, fused tests may lead to the fusion of cytoplasm but this does not also mean fusion of the nuclei. Each individual of the fused pair may preserve some independence, which may be reflected in the formation of double-sized chambers (as in *Planorbulina*, Le Calvez, 1938) or of double structures (such as the double tunnels in *Fusulina*, Wilde, 1965). However, the absence of nuclei fusion does not rule out common genetic regulation in sibling pairs.

In the specimen of *N. aspermontis* reported here, it could be argued that the initial growth after fusion of the two spirals indicates the maintenance of each individual's identity, and that the final stages of growth in a single spiral indicate common genetic regulation. However, similar growth patterns are found in normal specimens of multispiral species of

Nummulites (Ferràndez-Cañadell, 2012), in which at each growth step a chamber is added at the end of each spiral, and when two spirals collide, one of them usually overgrows the other. The same two patterns are apparent in the specimen of *N. aspermontis*. Furthermore, after 38 chambers of common growth the common spiral splits into two spirals for seven growth stages. Therefore, the growth pattern in this specimen does not aid in understanding the genetic relationship between the two individuals or how their genotypes interacted when their tests became fused. The development of a morphologically normal or abnormal test probably depends mainly on the relative geometric arrangement of the fused embryos. Tests with up to seven embryos may develop a normal test if they are arranged in the same plane [e.g., *Pseudophragmina* (*Proporocyclina*) *zaragosensis* in Cole, 1963, pl. 7, fig. 4], whereas tests with only two embryos may develop abnormal tests if they are arranged at a certain angle or with their apertures facing in opposite directions. On the other hand, abnormal growth might be related to differences between groups and in growth plan. For example, abnormal growth is common in twinned foraminifera with annular growth, either porcellaneous (e.g., *Orbitolites*) or hyaline (e.g., *Discocyclina*), whereas we have not found a single abnormal test in twin specimens of alveolinids, even in those with more than two embryos.

The two fused individuals in the specimen of *N. aspermontis* have megalospheric tests of similar size, differing only slightly in the number of chambers. Therefore, they probably came from the same mother after asexual reproduction, one of them growing faster and producing six chambers more than the other. In such a case they are either diploid schizonts with identical DNA, or they are haploid gamonts with different DNA. In the former case, growth would be regulated by identical DNA. In the second case, one of the haploid DNA would dominate (as in dominant alleles in diploid DNA); the same situation would occur if they were haploid gamonts from different mother individuals. The fourth possibility is that they were diploid schizonts from different mother individuals. In this case there would be competition between two different sets of DNAs, something that might prevent normal growth.

The easiest way to explain the common growth of this *Nummulites* specimen is that they were siblings (diploid schizonts with identical DNA), which would be consistent with their similar size before fusion and the rather common occurrence of twins in recent and fossil species. However, the possibility that they were haploid gamonts from the same mother or different mother individuals cannot be completely ruled out, and this would be the most likely situation for the different-sized fused individuals in the *Fusulina* specimen reported by Wilde (1965; Fig. 4).

Forcing Two Individuals to Fuse Their Tests

Because foraminifera repel each other and avoid contact when they meet, except during the very initial growth stages, fused adult individuals must be somehow forced to fuse their tests. One way would be by mechanically forcing the proximity of the individuals, in the way Schwab & Schwab-Stey (1980) experimentally forced the fusion of individuals of *Myxotheca arenilega*. A possible explanation for this mechanically forced fusion in nature could be ingestion by a metazoan, with possible fracture or partial etching in the digestive

tract, and the excretion of *Nummulites* specimens that are still alive, perhaps within a fecal pellet. Several metazoans ingest smaller and larger foraminifera, and an ingested foraminifer can survive passage through the digestive track of the metazoan, although its test may suffer some damage (e.g., Walker, 1971; Culver & Lipps, 2003; Goldbeck et al., 2005). The fused *Nummulites* individuals in this study are young, with tests ~1 mm in diameter, a size similar to that of smaller benthic foraminifera. After being ingested and excreted by an invertebrate or a fish, they could have remained attached to each other, possibly within a fecal pellet, in the same way as living *Rosalina floridana* (Cushman) occurs in fecal pellets of *Littorina littorea* (Linnaeus) (Walker, 1971); thus, when continuing their growth they built a common double test. This hypothesis could explain the merging of two individuals that would naturally repel each other when meeting.

Alternatively, adult fusion could be produced biochemically by environmental conditions restricting motility, together with a failure in the mechanism of allorecognition. For example, hypersalinity is known to hinder foraminiferal motility by perturbing the polymerization-depolymerization process of tubuline and actine (Stouff et al. 1999). Environmental stress, either natural or produced by anthropogenic pollution, produces abnormal growth in foraminifera. It could interfere with the foraminiferal allorecognition system and occasionally lead to test fusion of two adult individuals, especially if their motility is also affected.

Although there are many papers on growth abnormalities in foraminifera from polluted environments (see Alve, 1995, and Pati & Patra, 2012, for a review), most of the abnormalities deal with the size and shape of chambers and the thickness or ornamentation of the wall. Also double tests are found in foraminifera from zones with environmental stress, but clear examples of fusion of adult individuals have not been reported. Double tests can be produced by different causes, and are usually due to twining during reproduction. The rarity of reported fused adults can be an artifact due to the difficulty of obtaining adequate sections cutting the two proloculi. A systematic study of double tests by means of microCT in populations from stressed environments might reveal that adult fusion is actually not so rare, and that environmental stress can affect allorecognition in foraminifera.

On the other hand, the numerous studies on abnormalities published in the last two decades are centered on Recent species from shallow or restricted environments (paralic, estuarine, etc.), and mostly related to anthropogenic pollution. These shallow environments are easily affected by changes in salinity, which is known to produce test abnormalities, including double tests. The example of adult fusion in early Eocene *Nummulites* comes from a deeper environment. The fossil assemblage, dominated by orthophragminids (*Discocyclina* spp., *Nemkovella*, *Orbitoclypeus*, and *Asterocyclina*), together with *Assilina* and flat *Nummulites*, indicates a middle-outer platform environment, in which salinity could be excluded as the cause of environmental stress. Other possible causes of environmental stress in this context would be trophic levels (eutrophism) or turbidity (light), possibly affecting the foraminifera but most probably their symbiotic relationship with zooxanthellae. For example, an increase in nutrients input can affect the nitrogen cycle in the symbiotic relationship causing physiological stress to the foraminiferal host (Hallock, 2000). Nevertheless, it is difficult to relate this adult fusion in *Nummulites* to a concrete cause of environmental stress.

SUMMARY AND CONCLUSIONS

Double tests are usually interpreted as conjoined twins, although evidence of the presence of two or more embryos from a section is usually missing. Double tests are difficult to study using the traditional method of thin-sectioning, because the two (or more) embryos are arranged in different planes and cause growth in different directions. Computed tomography (CT) allows three-dimensional visualization of the external and internal structure of hollow objects and permits bi-dimensional visualization of unlimited planes cut through the scanned specimen. Therefore, CT scans of such teratological specimens are the only way to investigate their ontogeny.

Test fusion (twinning) is relatively common during schizogony, before the young are released from the brood chamber or cyst, where they are closely packed. However, typically one day after they are released the young repel each other when coming into contact, except in plastogamic species during reproduction. Test fusion between post-juvenile individuals of non-plastogamic species seems to be a rare phenomenon and very few examples are known in fossil species. To fuse their tests, either adult individuals have to be forced to do so or the allorecognition mechanisms must fail. A possible explanation for the merged tests of the two adult individuals of *Nummulites* described in this study is forced fusion in attached individuals after surviving ingestion and digestion by a metazoan. Alternatively, environmental stress could lead to a failure of the allorecognition mechanisms and/or the foraminiferal motility. In any case the frequency in which adult fusion occurs remains unknown. If it is really so rare as it seems, the former explanation could explain the few known examples. If a systematic study of double tests reveals that adult fusion is more frequent, the disturbance of allorecognition and motility by environmental conditions would then seem to be the more probable cause.

Because the tests of the two fused individuals of *N. aspermontis* reported here are of a similar size and growth stage, they were probably sister clones of the same mother individual. In that case allorecognition mechanisms could be not as strong as in haploid gamonts from the same or different mother individual. However, this latter possibility cannot be completely ruled out. The behavior of merged individuals with identical DNA (sister clones), or with different haploid or diploid DNA could only be solved experimentally, after artificially adhering their tests.

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REFERENCES

- Abrard R. Développements aberrants chez des nummulites. Comptes rendus de l'Académie des Sciences, Paris. 1945; 220(22):786–787.
- Abrard R. Individus tératologiques de *Nummulites* d'Aquitaine. Compte Rendu Sommaire des Séances de la Société Géologique de France. 1951; 5-6:95, 96.

- Alve E. Benthic foraminiferal responses to estuarine pollution: A review. *Journal of Foraminiferal Research*. 1995; 25:190–203.
- Arnold, ZM. Biological observations on the foraminifer *Spiroloculina hyalina* Schulze. 1964. p. 1-93. University of California Publications in Zoology, v. 72
- Bradley JS. A teratoid *Parafusulina*. *Journal of Paleontology*. 1956; 30:303–304.
- Briguglio A, Benedetti A. X-ray microtomography as a tool to present and discuss new taxa: the example of *Risananeiza* sp. from the late Chattian of Porto Badisco. *Rendiconti Online Società Geologica Italiana*. 2012; 21:1072–1074.
- Briguglio A, Metscher B, Hohenegger J. Growth rate biometric quantification by X-ray microtomography on larger benthic foraminifera: three-dimensional measurements push nummulitids into the fourth dimension. *Turkish Journal of Earth Science*. 2011; 20:683–699.
- Briguglio, A.; Wöger, J.; Wolfgring, E.; Hohenegger, J. Changing investigation perspectives: methods and applications of computed tomography on larger benthic foraminifera. In: Bernard, J.; Kitazato, H., editors. *Experimental Approaches in Living Foraminifera: Collection, Maintenance and Experiments*. Springer; Tokyo: 2014. p. 55-70. Environmental Science and Engineering Series Chapter 4
- Carpenter WB. Researches on the Foraminifera. Part I. Containing general introduction, and monograph on the genus *Orbitolites*. *Philosophical Transactions of the Royal Society of London*. 1856; 146:181–236.
- Cecchia-Rispoli G. Sopra alcune Alveoline eoceniche della Sicilia. *Palaeontographia Italica*. 1905; 11:147–165.
- Cole WS. Variability in embryonic chambers of *Lepidocyclina*. *Micropaleontology*. 1960; 6:133–140.
- Cole WS. Illustrations of conflicting interpretations of the biology and classification of certain larger foraminifera. *Bulletins of American Paleontology*. 1963; 46:1–63.
- Culver, J.; Lipps, JH. Predation on and by Foraminifera. In: Kelley, PH., et al., editors. *Predator-Prey Interactions in the Fossil Record*. 2003. p. 7-32. Topics in Geobiology, v. 20, pt. I
- de la Harpe PG. Étude des Nummulites de la Suisse et révision des espèces éocènes des genres *Nummulites* et *Assilina*. Première partie: Mémoires de la Société Paléontologique Suisse. 1881; 7:4–104.
- De Zanche V. Osservazioni sulla patologia di nummuliti ed assiline e sul singolare stato di conservazione di alveoline nei pressi di Albanello in valle del Chiampo, Vicenza. *Memorie degli Istituti di Geologia e Mineralogia dell'Università di Padova*. 1966; 25:1–17.
- Douvillé H, O'Gorman G. L'Éocène du Béarn. *Bulletin de la Société géologique de France*. 1929; 29:329–390. ser. 4.
- Elgar MA, Crozier ER. Animal allorecognition systems: how to get to know yourself. *Trends in Ecology and Evolution*. 1989; 4:288, 289.
- Ferrández-Cañadell C. A new, ribbed species of *Nemkovella* Less, 1987 (Discocyclinidae), and discussion of the genus *Actinocyclina* Gümbel, 1870. *Journal of Foraminiferal Research*. 1997; 27:175–185.
- Ferrández-Cañadell C. Multispiral growth in *Nummulites*. Paleobiological implications. *Marine Micropaleontology*. 2012; 96-97:105–122.
- Goldbeck EJ, Houben C, Langer MR. Survival of foraminifera in the gut of holothuroids from Elba Island (Mediterranean Sea). *Revue de Micropaléontologie*. 2005; 48:169–174.
- Hallock P. Symbiont-bearing foraminifera: harbingers of global change? *Micropaleontology*. 2000; 46(suppl. 1):95–104.
- Heron-Allen E. Contributions to the study of the bionomics and reproductive processes of the Foraminifera. *Philosophical Transactions of the Royal Society of London, Series B*. 1915; 206:227–279.
- Hohenegger, J.; Briguglio, A. Methods for estimating individual growth of foraminifera based on chamber volumes. In: Bernard, J.; Kitazato, H., editors. *Experimental Approaches in Living Foraminifera: Collection, Maintenance and Experiments*. Springer; Tokyo: 2014. p. 29-54. Environmental Science and Engineering Series Chapter 3
- Hottinger L. Illustrated glossary of terms used in foraminiferal research. *Carnets de Géologie*. 2006:1–126. Memoir 2006/02 (CG2006_M02).

- Hughes RN, Manríquez PH, Morley S, Craig SF, Bishop JDD. Kin or self-recognition? Colonial fusibility of the bryozoan *Celleporella hyalina*. *Evolution and Development*. 2004; 6:431–437. [PubMed: 15509225]
- Jensen P. Über individuelle physiologische Unterschiede zwischen Zellen der gleichen. *Archiv für die gesamte Physiologie des Menschen und der Tiere*. 1895; 62:172–200.
- Kahler F. Beobachtungen über Lebensweise, Schalenbau und Einbettung jungpalaözoischer Großforaminiferen (Fusuliniden). *Facies*. 1988; 19:129–170.
- Keckskeméti T. Patológikus jelenségek nummuliteszeken. *Földtani Közlöny, Bulletin of the Hungarian Geological Society*. 1962a; 92:209–219.
- Keckskeméti T. Pathologische Erscheinungen an Nummuliten. *Annales Historico-Naturales Musei Nationalis Hungarici, Pars Mineralogica et Palaeontologica*. 1962b; 54:73–84.
- Kloos DP. Parents and broods of *Sorites orbiculus* (Forskål), a biometric analysis. *Journal of Foraminiferal Research*. 1984; 14:277–281.
- Kobayashi F, Altiner D. Fusulinoidean faunas from the Upper Carboniferous and Lower Permian Platform Limestone in the Hadim Area, central Taurides, Turkey. *Rivista Italiana di Paleontologia e Stratigrafia*. 2008; 14:191–232.
- Krüger R, Röttger R, Lietz R, Hohenegger J. Biology and reproductive processes of the larger foraminiferan *Cycloclipeus carpenteri* (Protozoa, Nummulitidae). *Archive für Protistenkunde*. 1997; 147:307–321.
- Langer MR, Makled WA, Pietsch SJ, Weinmann A. Asynchronous calcification in juvenile megalospheres: an ontogenetic window into the life cycle and polymorphism of *Peneroplis*. *Journal of Foraminiferal Research*. 2009; 39:8–14.
- Le Calvez J. Embryons à cinq loges de *Planorbulina mediterraneensis* (d'Orb.) et trimorphisme de cette espèce. *Bulletin de la Société zoologique de France*. 1934; 59:284–290.
- Le Calvez J. Recherche sur les foraminifères, I. Développement et reproduction. *Archives de Zoologie Experimentale et Generale, Paris*. 1938; 80:163–333.
- Marchesini E, Facca GC. Sulla variabilita di *Nummulites fichteli* Michelotti. *Paleontographia Italica*. 1940; 40:39–65.
- Meriç E, Avsar N, Görmüş M. Twin forms in recent benthic foraminifera from the northern Aegean Sea and western Black Sea regions (Turkey). *Revue de Paléobiologie*. 2001; 20:69–75.
- Meriç E, Yoke MB, Nielsen JK, Görmüş M, Avsar N, Dinger F. Abnormal formations in peneroplids: *Peneroplis-Coscinospira* togetherness. *Anales de Biología*. 2008; 30:1–7.
- Meriç E, Yoke MB, Avsar N, Bircan C. A new observation on abnormal development in benthic foraminifera: *Peneroplis pertusus* (Forskål)-*Peneroplis planatus* (Fichtel and Moll) togetherness. *Anales de Biología*. 2012; 34:41–46.
- Mukhopadhyay SK. Plastogamy and its early morphological indication in *Nummulites boninensis* Hanzawa from the Middle Eocene of Cambay Basin, India. *Revue de Paléobiologie*. 2003; 22:231–242.
- Mukhopadhyay SK. Conjoined tests of *Nummulites* from the Paleogene of the Cambay Basin, India, and their possible origin. *Journal of Foraminiferal Research*. 2007; 37:41–45.
- Pati P, Patra PK. Benthic foraminiferal responses to coastal pollution: a review. *International Journal of Geology, Earth and Environmental Sciences*. 2012; 2:42–56.
- Pavlovic R. Patologija nummulitin. *Geologija Razprave in Porocila*. 1976; 19:83–93.
- Polovodova I, Schönfeld J. Foraminiferal test abnormalities in the western Baltic Sea. *Journal of Foraminiferal Research*. 2008; 38:318–336.
- Popescu-Voitești I. Abnormale Entwicklung bei Nummuliten. *Beiträge zur Palaöontologie und Geologie Österreich-Ungarns und des Orients*. 1908; 21(3/4):211–214.
- Preobrazhenskaya TV, Tarasova TS. Sexual reproduction of the foraminiferan *Planoglabratella opercularis* (d'Orbigny, 1839) in nature. *Russian Journal of Marine Biology*. 2004; 30:323–327.
- Regè R. Nummuliti ed Orbitoidi di alcune località istriane. *Atti della Società Italiana di Scienze Naturali*. 1916; 55:193–234.

- Rhumbler L. Die Doppelschalen von *Orbitolites* und anderen Foraminiferen, vom entwicklungsmechanischen Standpunkt aus betrachtet. Archiv für Protistenkunde. 1902; 1:193–296.
- Rhumbler, L. Die Foraminiferen (Thalamophoren) der Plankton-Expedition Zugleich Entwurf eines natürlichen Systems der Foraminiferen auf Grund selektionistischer und mechanisch-physiologischer Faktoren. Lipsius und Tischer; Kiel: 1911. p. 331
- Richardson-White S, Walker SE. Diversity, taphonomy and behavior of encrusting foraminifera on experimental shells deployed along a shelf-to-slope bathymetric gradient, Lee Stocking Island, Bahamas. Palaeogeography, Palaeoclimatology, Palaeoecology. 2011; 312:305–324.
- Röttger, R.; Spindler, M. In: Schafer, CT.; Pelletier, BR., editors. Development of *Heterostegina depressa* individuals (Foraminifera, Nummulitidae) in laboratory cultures; First International Symposium on Benthonic Foraminifera of Continental Margins; 1976; p. 81-87. Part A: Ecology and Biology; Maritime Sediments Special Publication No. 1
- Schaub H. *Nummulites* et *Assilina* de la Téthys paléogène. Taxinomie, phylogénèse et biostratigraphie. Mémoires Suisses de Paléontologie. 1981; 104-106:1–236.
- Schultze, MS. Über den Organismus der Polythalamien (Foraminiferen). Wilhem Engelman; Leipzig: 1854. p. 68
- Schwab D, Schwab-Stey H. Induced cell fusion in foraminifera. Protoplasma. 1980; 102:141–146.
- Serra-Kiel J, Hottinger L, Caus E, Drobne K, Ferràndez C, Jauhari AK, Less G, Pavlovec R, Pignatti J, Samsó JM, Schaub H, Sirel E, Strougo A, Tambareau Y, Tosquella J, Zakrevskaya E. Larger foraminiferal biostratigraphy of the Tethyan Paleocene and Eocene. Bulletin de la Société géologique de France. 1998; 169:281–299.
- Spiegler D. Abnormale Entwicklungserscheinungen an Nummuliten vom Fundpunkt Brandhorst bei Bünde. Geologie. 1958; 7:1058–1065.
- Stouff V, Debenay J-P, Lesourd M. Origin of double and multiple tests in benthic foraminifera: observations in laboratory cultures, environments. Marine Micropaleontology. 1999; 36:189–204.
- Thompson, ML. Fusulinacea. In: Moore, RC., editor. Treatise on Invertebrate Paleontology, Part C, Protista 2. Vol. 2. Geological Society of America and University of Kansas Press; Lawrence: 1964. p. C358-C436.
- Walker DA. Etching of the test surface of benthonic foraminifers due to ingestion by the gastropod *Littorina littorea* Linné. Canadian Journal of Earth Science. 1971; 8:1487–1491.
- Wilde GL. Abnormal growth conditions in fusulinids. Cushman Foundation for Foraminiferal Research. 1965; 16:121–124.

Figure 1.

1 SEM external views of the fused specimen of *Nummulites aspermontis*. Note the involute lamellation in 1c. Scale bars = 1 mm. **2** Axial sections imaged by computed microCT, showing the two proloculi followed by 2–3 whorls of independent growth. **3** Three-dimensional axial section imaged by computed microCT, showing the initial independent spirals at 81° followed by enveloping common chambers. **4** Three-dimensional double equatorial section imaged by computed microCT, showing the initial spirals with >two whorls of independent growth before merging of the two tests.

Figure 2.

MicroCT equatorial sections of the two individuals, A (**1a**) and B (**2a**), in Figure 1, showing reconstruction of their tests at the time of fusion, when individual A (**1b**) had 30 chambers and individual B (**2b**) had 36 chambers.

Figure 3.

Three-dimensional models of the two individuals at different ontogenetic stages based on reconstruction of chamber lumina by means of microCT. Arrows show the growth direction of the individuals. **1** Contact between the two individuals at chambers 30 of A (A30) and 36 of B (B36). **2** Overgrowth of individual B on individual A. Chamber 44 of specimen B (B44) has three alar prolongations, two in the external lateral part of each individual and one in between. From here on the spiral splits into two parts, one of them very short, with five chambers, growing on specimen A (with chambers B44-1 and B44-2 growing on A35–A38),

and the other one growing normally following its former whorls. **3** Frontal collision of the individual spirals. Chamber A54 frontally collides with chamber B60 and a combined chamber (here called A55-B61) is generated. The following chambers (here called AB1–AB4) follow the A spiral. At the end of this whorl the spiral splits again into two parts above chamber A55–B61 and grows simultaneously in two spirals, towards chambers B59 and AB3.

Figure 4.

Interpretation of the fused adult individuals of *Schwagerina* sp. illustrated in Wilde (1965, pl. 19, fig. 5). The tests of the two individuals seem to be attached by the broken chambers 33–35 of one individual and 55–56 of the other (dashed white line). After fusion, the smaller individual (light gray) seems to have built only two chambers, with growth being dominated by the larger individual (dark gray), which grew to envelop both tests (white).

Table 1

Main parameters for microCT using a Skyscan 1163.

Camera Pixel size	50.0 μm
Kv	80
uA	100
Rotation step	0.15°
Image pixel size	5.34511 μm
Exposure	650 ms
Frame Averaging	5
360 rotation	No
Filter	Al 1.0 mm